Aquaculture of Sponges for Production of Bioactive Metabolites

A thesis submitted in partial fulfilment
of the requirements for
the Degree of
Doctor of Philosophy in Zoology
in the University of Canterbury
by

A R Duckworth

University of Canterbury
2000

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Acknowledgements

After nine years of university study and 3½ years of PhD work my student days are over. I thank my supervisors Drs Chris Battershill, Dave Schiel and Prof Pat Bergquist for critically reading this thesis and for their advice. I also thank Drs Rob Murdock, Clive Howard-Williams, Vicky Webb and Mike Beardsell for reading parts of this thesis.

Apart from a few laboratory-based experiments, all of the research for this PhD was done underwater. Anyone who has dived in the waters off Wellington, particularly on the South Coast, will know that it is often a very cold, windy and a generally unpleasant experience. Therefore, a huge thank you to the following who have helped me with my diving: Chris Woods, Pete Notman, Phil James, Mike Page, Rob Stewart, Dean Stotter, Jeff Foreman, Dick Singleton, John Hunt, Owen Anderson, Steve Mercer and Ray Mulder. To Chris and Pete, I simply could not have done this PhD with out your help and great support - thanks! Overall, I entered the briny-depths 401 times, and spent 280 hours underwater or 7½ working weeks being wet.

I would also like to thank NIWA for providing logistical support during my study. This PhD was done under the University of Canterbury/NIWA Centre of Excellence in Aquaculture and Marine Ecology programme and was funded by the Foundation for Research, Science and Technology.

Thanks also to the many good friends and flatmates who livened-up my time in Wellington and diverted my thoughts away from study, especially Evelyn, Lucie, Anton, Kelly, Graeme, Hamish, Nigel, Rebecca, Julian and Stu.

Finally, it is imperative! that I thank my mum, dad and sister Kim for their support throughout my varsity days and for the many words of encouragement during my PhD. Perhaps through reading this thesis you can appreciate, if not understand, the long process of studying and working in marine science.

Abstract

Sponges have proved to be the best source among marine organisms of biologically active metabolites for use as drugs or biomedical tools. If successful in clinical trials, bioactive metabolites will be needed in vast quantities, but most sponges contain only trace amounts of them. Of the supply methods currently being examined, aquaculture is considered to be the most cost-effective or perhaps the only method to guarantee sufficient supplies of some sponge metabolites. Two factors restricting the commercial development of sponge aquaculture are a poor understanding of how the environment affects the growth, survival and metabolite biosynthesis of sponges, and the lack of a farming structure that can supply sufficient quantities of bioactive metabolites. This study examined these factors focusing on two species, *Latrunculia brevis* and *Polymastia croceus*, both of which contain metabolites with biomedical application.

For three years the population dynamics, growth and bioactivity (measure of metabolite biosynthesis) of wild *L. brevis* and *P. croceus* were examined to further our knowledge about sponge ecology and also to provide information to help develop good methods and procedures to farm sponges. For both species, survival of adult sponges was high in all seasons, while juvenile sponges had poor survival. Recruitment of *L. brevis* occurred in all seasons indicating that it is reproductively active throughout the year. *P. croceus* recruited mostly in autumn, this observation supports previous work that found the sponge to be reproductively active in summer and early autumn only. For both species, growth rates varied greatly between individuals and were unaffected by sponge size within the range examined. Sponges generally grew during winter and spring as the water temperature rose and shrank during summer and autumn as the water temperature fell. This growth pattern may relate to seasonal variation in food abundance, and for *P. croceus* it may result also from seasonal differences in reproductive investment. After 2 years, *L. brevis* and *P. croceus* had on average, halved and doubled in size, respectively. This indicates that wild sponges generally grow slowly and can also shrink in size. *L. brevis* showed a seasonal pattern of bioactivity, being most active in spring possibly to prevent the surface overgrowth of fouling organisms. *P. croceus* had no seasonal pattern of bioactivity, but individuals were either very active or inactive. These patterns of bioactivity may indicate an

optimal defence strategy whereby sponges increase metabolite synthesis when they are most required. The bioactive metabolites in both species probably aid in competitive interactions and prevent predation and biofouling.

The major environmental factors that are likely to influence the growth, survival and metabolite biosynthesis of farmed sponges and thus directly affect the success of a farming operation are season, exposure or water movement and depth. The effect of these environmental factors were examined in a series of short-term transplant experiments. Both survival and growth of *L. brevis* were greatest in winter when the water temperature was lowest, which probably reduces stress during transplanting. For *P. croceus*, survival was similar in winter, spring and autumn, while growth was greatest in spring probably because of greater food availability. Therefore, the response to the farming season can vary greatly between sponge species. During the summer transplant the toxic alga *Gymnodinium brevisulcatum* bloomed, killing most farmed explants. This highlights the danger to sponge aquaculture of adverse stochastic events that cannot be planned for or controlled. Growth of *L. brevis* and *P. croceus* generally increased as exposure increased thus showing clearly that although sponges are active suspension feeders, they rely greatly on the passive flow of water to provide food. The depth range (5-15m) examined in this study had no overall effect on the growth or survival of either *L. brevis* or *P. croceus*. Explants of both species farmed in similar environmental conditions varied greatly in growth. For both species, farming promoted greater synthesis of bioactive metabolites, which may indicate an optimal defence strategy. A reciprocal transplant experiment between northern and central New Zealand indicated that *P. croceus* could be transplanted great distances and survive. However, growth of relocated explants is reduced until they adjust to their new environment.

A series of experiments was done to develop farming structures suitable for sponge aquaculture for metabolite production. Four general methods were examined: farming explants inside mesh structures, attached to substrate, with rope threaded through them and rope wrapped around them. Each was further divided into several specific methods examining the effects of various mesh sizes and rope materials. Most methods were found to be unsuitable because the farmed explants did not attach to the substrate but instead moved away from it and dislodged themselves. The two methods that showed the most potential for farming sponges, in terms of good growth, survival

and metabolite biosynthesis, were threaded PVA rope and individual mesh bags with large holes and thin strand. These were developed into "rope" and "mesh" arrays.

For nine months, *L. brevis* and *P. croceus* were farmed in rope and mesh arrays and harvested at different times. Harvesting involved the removal of new tissue growth leaving the explant "core" behind to regrow. The water temperature at the time of harvesting greatly affected the survival of *L. brevis* but not *P. croceus*. This supports the results of the short-term transplant experiments. Growth after harvesting was similar between harvested and non-harvested explants, indicating that healing of cut tissue and reorganisation of the canal system is not a drain on resources. This experiment showed that sponges can have very high growth rates. For example, explants of *L. brevis* and *P. croceus* in one treatment had grown by an average of 950% and 740% of their initial volume, respectively, in six months. Both rope and mesh arrays were found to be good farming structures, but differing patterns of growth and survival indicated that the two arrays are most suited for a particular type of sponge depending on its tissue structure. Rope arrays should be used to farm firm sponges such as *P. croceus* that can survive the threading process, while mesh arrays are best for farming soft, fleshy sponges like *L. brevis* that can grow quickly through the mesh strands. In some treatments, overall tissue yields were double the initial transplanted weight. As before, farmed sponges were generally more bioactive than wild sponges.

An experiment to examine whether harvesting wild sponge populations is a suitable alternative method of supplying bioactive metabolites found that individuals of *L. brevis* and *P. croceus* could survive after removal of ≥90% of their biomass. Tissue regrowth was rapid and it was estimated to take between 1-4 years for individuals to grow back to their pre-harvested size. Although this suggests that harvesting wild populations of *L. brevis* and *P. croceus* can be an alternative method of metabolite supply, it is limited because of the relative scarcity of the sponges in the natural environment.

This study examined the effect of different environments on the growth, survival and metabolite biosynthesis of sponges and developed methods and structures suitable for farming sponges. The high tissue yields from some treatments and the

elevated bioactivity of farmed explants suggests that sponge aquaculture is a viable commercial method of supplying bioactive metabolites.

Chapter 1. General Introduction

1.1 Background to the study

For thousands of years mankind has relied on nature to supply medicines to cure diseases. These medicines were predominately derived from terrestrial plants (Kelecom 1991, Carté 1993, 1996) and worked because the bioactive metabolites synthesised by plants possibly as a defence against predation (Fenical 1996) also have medicinal properties. In developing countries, traditional medicines prepared from plant products still form the basis of their primary health care (Cragg et al. 1997 and references therein). Bioactive metabolites obtained from plants are also important in developed countries, and are used extensively by the pharmaceutical industry as drugs and biochemical tools (Davidson 1995, Cragg et al. 1997). For example, one quarter of the anticancer drugs used today contain plant bioactive metabolites (Davidson 1995).

Unfortunately, even with the plethora of drugs available today millions of people still die from disease. Each year, 7 million people die from respiratory infections caused by bacteria (Natural History 1999), while cancer kills half a million people annually in the USA alone (Landis et al. 1998). Not only are disease causing organisms evolving quickly, so that diseases treatable today may not be treatable tomorrow (Fenical 1996, 1997, Shu 1998), but also longer life expectancy increases the chance of developing cancer. The World Health Organisation predicts by the year 2020 the number of new cancer sufferers will increase from the current 10 million a year to 20 million (Irwin 1998).

Because of the rich and largely unexplored biodiversity of the marine environment, mankind looked to the oceans in the search for new drugs to combat disease. The first marine drug discovery programmes started in the 1970's (Fenical 1997) and thousands of bioactive metabolites from a wide range of marine organisms have since been screened (Munro et al. 1994). Screening has concentrated on finding new anticancer, antiviral, anti-inflammatory and immunosuppressive compounds, and several marine metabolites are currently in clinical and pre-clinical trials as potential new drugs.

Of all the marine fauna, sponges are the best source of metabolites with biomedical potential (Ireland et al. 1988, 1993) and produce many of the metabolites currently being tested in clinical trials. Current examples include, the immunosuppressive metabolite discodermolide from the Caribbean sponge *Discodermia dissoluta* (Gunasekera et al.

1990) and the anticancer agent isohomohalichondrin B isolated from the New Zealand sponge *Lissodendoryx* n. sp. (Munro et al. 1999). The market value of a marine bioactive metabolite is potentially huge if it is successful in all trials and is approved as a drug. For example, the anticancer metabolite bryostatin-1 from the bryozoan *Bugula neritina* is estimated to be worth US$1 billion per year (Pain 1998).

Up to one kilogram of a bioactive metabolite is required for drug development alone (Cragg et al. 1997). Unfortunately, most sponges contain only trace amounts of bioactive metabolites (Schmitz et al. 1993) and the need for large quantities for trials has at times resulted in the near extinction of a population or a species (Anderson 1995). The supply issue becomes a much more serious problem if the sponge metabolite is used in commercial drug manufacture (Shimizu 1995, Dumdei et al. 1998, Munro et al. 1999). It is therefore essential to develop methods to guarantee the supply of metabolites for the pharmaceutical industry both for drug development and long-term commercial production. Supply methods currently being developed include harvesting from wild populations, chemical synthesis, cell culture, genetic engineering, and aquaculture.

Harvesting from wild populations to obtain bath sponges has been practised for thousands of years and capitalises on the ability of many sponges to regenerate lost tissue (Storr 1957). Unfortunately, harvesting is susceptible to overfishing and disease outbreaks (Vacelet et al. 1994). In addition, harvesting is not a viable option for many species because metabolite demand will exceed what the wild population can provide (Pain 1996). If isohomohalichondrian B becomes a commercial drug, for example, it is estimated that 5000 tonnes of *Lissodendoryx* n. sp. will be needed each year to supply the market (Munro et al. 1999), yet the total wild population is only ~290 tonnes (Dumdei et al. 1998). Although harvesting may be sustainable for abundant and fast-growing species, many sponges have a threshold to damage and if too much tissue is removed, by predators for example, the sponge cannot recover (Dayton 1979, Shield and Witman 1993). Therefore, before a species is commercially harvested from wild populations it is important to determine how much tissue can safely be removed from an individual sponge. In addition, examining the rate of regeneration of lost biomass will indicate how often a population can be harvested.

Chemical synthesis, where the metabolite is manufactured in the laboratory, is an alternative method of supply. Unfortunately, the complexity and size of many bioactive metabolites often makes them difficult to manufacture (Pain 1996) and so far it has been

very difficult to adapt bench scale synthesis to large scale economic production (Cragg et al. 1997). For this reason, many promising leads have been abandoned by drug companies.

Cell culture, where the cell responsible for metabolite biosynthesis is cultured, is presently being developed (Pomponi and Willoughby 1994). However, researchers have encountered many problems (Ilan et al. 1996), particularly in enabling sponge cells to carry on dividing (Pain 1996), and the full commercial development for this supply option is possibly decades away. Some bioactive metabolites are not produced by the sponge itself but are instead biosynthesised by a symbiont (Bergquist and Wells 1983). However, the isolation of the symbiont has proved difficult, thus preventing its individual culture.

Genetic engineering bypasses the difficult culturing of sponge cells by splicing the part of the genome that codes for the biosynthetic pathway into a more easily cultured organism, such as a bacterium. As with cell culture, development of such techniques is just beginning and the commercial use of this method is likely to be decades away (Munro et al. 1999).

Aquaculture, where sponge pieces or explants are farmed, is another supply option, and based on current understanding it is considered to be the most cost-effective or perhaps only method to guarantee sufficient supplies of some sponge metabolites (Shimizu 1995, Munro et al. 1999). Even if the target metabolite is produced by a symbiont, aquaculture of the sponge plus symbiont may still be the best method of supplying that metabolite.

Sponge aquaculture for bath sponges was first tested in the mid 1800s and resulted from environmental concerns of over-harvesting wild sponge populations (Crawshay 1939). These early attempts failed, however, mainly because no suitable structure was found for growing sponges. Later, Moore (1908a) found that explants of bath sponges grew well when attached to concrete discs or when threaded with wire so that they hung in mid-water. These farming structures were also used, with some modification, by Crawshay (1939) and the Japanese before World War II (Cahn 1948) to grow bath sponges. The development of cheap synthetic sponges during World War II removed the need to farm sponges (Bergquist and Tizard 1969), and farming studies stopped. However, the increasing demand for natural bath sponges, and concerns about over-fishing, have resulted again in studies to examine the potential of bath sponge aquaculture (Verdenal and Vacelet 1990, Pronzato et al. 1999).

In the past decade, a few studies have tested the farming of sponges that have metabolites of interest to the pharmaceutical industry (Battershill and Page 1996, Duckworth et al. 1997, Dumdei et al. 1998, Munro et al. 1999). Although these studies were exploratory in nature, they produced some promising results. Duckworth et al. (1997) discovered that explants of *Raspailia agminata* farmed in mesh bags could double in weight in 9 months. Battershill and Page (1996) found that some *Lissodendoryx* n. sp. explants farmed in scallop lanterns could grow by 5000% of their initial size in a few months. Both studies grew explants in mesh structures, a method probably unsuitable for the production of bath sponges because the mesh could interfere with the final shape of the sponge and thus reduce its market value (Storr 1964, Bergquist and Tizard 1969). Because sponge aquaculture for metabolite production is unconstrained by the shape of the sponges, there is considerable flexibility in developing new farming structures where sponge tissue is required only for metabolite production.

To farm sponges for metabolite production successfully requires a good understanding of how the environment affects the growth, survival and metabolite biosynthesis of sponges. One finding common to many ecological studies is that seasonal cycles of water temperature can affect the growth (Stone 1970, Elvin 1976, Barthel 1986, Turon et al. 1998), survival (Johnson 1979, Fell and Lewandroski 1981, Frost et al. 1982) and metabolite biosynthesis (Green et al. 1990, Turon et al. 1996, Swearingen and Pawlik 1998) of sponges. However, apart from the study by Duckworth et al. (1997) who found that transplanted explants of *Psammocinia hawere* grow and survive better in winter than in summer, the effect of seasonal cycles of water temperature on farmed sponges is unknown. Another factor that is probably important for farming sponges, but which has received little attention, is the effect of water movement or exposure. Even though sponges are active suspension feeders and capable of generating their own water movement, they rely greatly on the passive flow of water to provide food (Vogel 1974). This indicates that water movement will affect sponge growth, but this relationship has been examined in few studies and with seemingly conflicting results. While Wilkinson and Vacelet (1979) observed that growth of transplanted sponges generally increased as exposure increased, Duckworth et al. (1997) and Leichter and Witman (1997) observed that growth of transplanted sponges in their studies was poorest overall in the most exposed treatments. Before sponge aquaculture can be considered a viable method of

metabolite supply, it is important to determine the effect of both season and exposure on the farming responses of sponges.

A further contributing factor that is restricting the development of sponge aquaculture for metabolite production is that there is presently no farming structure suitable for the large-scale commercial aquaculture of sponges for metabolite production (Shimizu 1995, Osinga et al. 1998). It is important to develop a farming structure before sponge metabolites are needed in commercial quantities for drug production to guarantee supply and to allow immediate commencement of farming. Thus, the main aims of this thesis are:

(1) to examine how the environments affects the growth, survival and metabolite biosynthesis of sponges and,

(2) to develop farming structures that are suitable for the large-scale commercial aquaculture of sponges for metabolite production.

These two aims are examined in four experimental chapters. The growth, population dynamics and metabolite biosynthesis of wild sponges are examined over several years (Chapter 2) to provide information on seasonal effects and patterns. The influence of the farming season, exposure and depth on the growth, survival and metabolite biosynthesis of farmed sponges are examined in Chapter 3. Chapter 4 describes a series of experiments done to develop farming structures for commercial sponge aquaculture. The knowledge gained and farming structures developed from the previous chapters are examined further in Chapter 5 to determine if large-scale production of bioactive metabolites is possible. In addition to research on sponge aquaculture, the effect of harvesting wild sponge populations is examined to determine whether harvesting is an alternative and commercially viable method of supplying bioactive metabolites (Chapter 6)

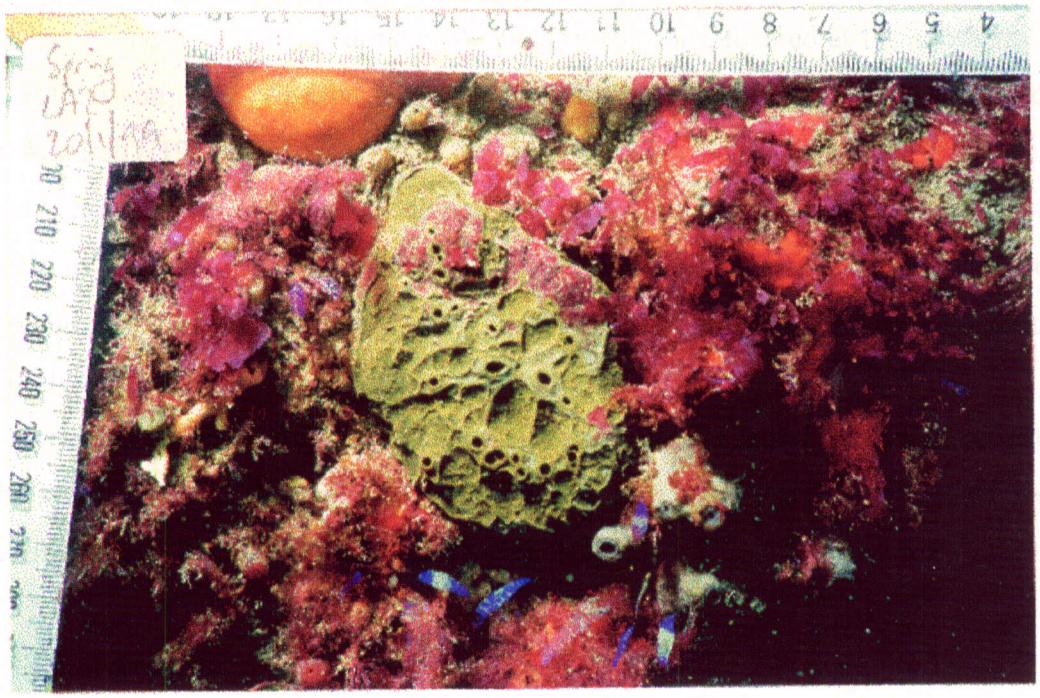
1.2. Study organisms

This thesis focused on two locally abundant sponge species that contain metabolites of biomedical interest. One species was *Latrunculia brevis* (Ridley and Dendy 1886) (Fig1.1a) which is a green massive sponge found throughout New Zealand, usually in exposed areas (Battershill and Bergquist 2000). The taxonomy of this species is currently being investigated. A recent study examining the genetic structure of *L. brevis* sourced from populations throughout New Zealand indicated that there are several species to which this name has been assigned (Miller et al. 1999). The genetic study also indicated

that there is only one species in Wellington which was the source population of sponges in this study. Until the proper nomenclature is decided, the name *L. brevis* will be used. The bioactive metabolite of medicinal interest in *L. brevis* is the pigment molecule discorhabdin which has strong antitumor and antimicrobial properties (Perry et al. 1988, Lill et al. 1995). So far, 15 types of discorhabdin have been discovered in *L. brevis*, each with subtle differences in bioactivity (Northcote, Victoria University of Wellington, personal communication). Discorhabdins are biosynthesised by *L. brevis* and not by a symbiotic micro-organism (Lill et al. 1995). The second species is *Polymastia croceus* (Kelly-Borges and Bergquist 1997) (Fig. 1.1b) which is an orange massive sponge commonly found subtidally throughout New Zealand. Some aspects of its ecology, particularly its reproductive biology, have been examined in northern New Zealand populations (Ayling 1980, Battershill and Bergquist 1990). The bioactive metabolite of medicinal interest has not been fully identified but it does have strong antitumor properties (National Cancer Institute, personal communication). Given the histology of *P. croceus* and general absence of symbionts it is most likely that the target metabolite is of sponge origin (Bergquist, University of Auckland, personal communication).

Mycale sp., *Polymastia massilis* and *Raspailia agminata* also sponges containing metabolites with biomedical potential, were included in some experiments. *Mycale* sp. is a brown encrusting sponge that contains the metabolites mycalamide and pateamine. Mycalamide has strong anticancer properties (Burres and Clement 1989) while pateamine may be used in biomedical research as a probe to examine cellular functions (West, Victoria University of Wellington, personal communication). *Polymastia massilis* (Carter) is a brown massive sponge found subtidally, generally in sheltered locations (Kelly-Borges and Bergquist 1997). The metabolite of interest is presently undescribed but it has attracted much interest from the National Cancer Institute (Battershill, Australian Institute of Marine Science, personal communication). *Raspailia agminata* (Hallman) is a dark brown massive or encrusting sponge (Bergquist 1970) and one metabolite that it contains is okadaic acid which is used in biomedical research to examine the growth of tumours (Yatsunami et al. 1992).

(a) *Latrunculia brevis*



(b) *Polymastia croceus*

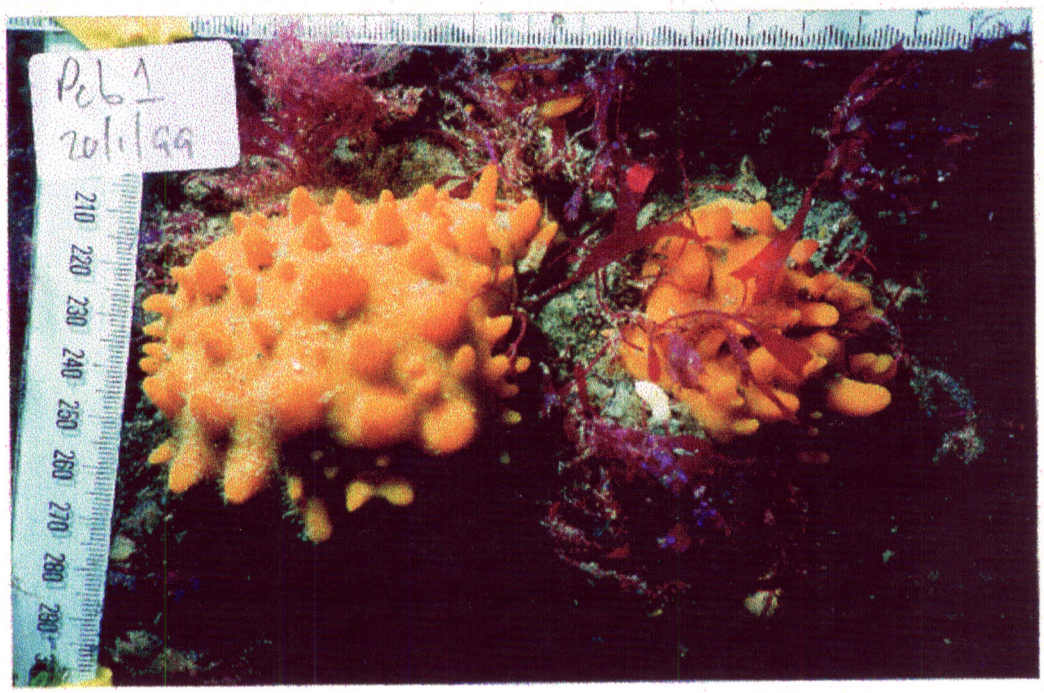


Figure 1.1. Photographs of *Latrunculia brevis* and *Polymastia croceus*. The photos also show the ruler used for measuring sponge size and the identification tag (top left corner).

1.3. Study locations

Most of the research was done in Wellington Harbour, located at the southern end of the North Island, New Zealand (Fig. 1.2). Wellington Harbour has an area of 76km^2 (British Admiralty 1971) and an average depth of 14m (Heath 1977). While the harbour entrance is exposed to both prevailing north-westerly winds and southerly storms, the inner harbour includes both moderately exposed and sheltered conditions (Northcote 1998). Wellington Harbour, therefore, provides a great range of environmental conditions for the study of sponge aquaculture.

Latrunculia brevis, *Polymastia croceus* and *Polymastia massilis* were all collected from the south coast of Wellington, while *Mycale* sp. and *Raspailia agminata* were collected from Cape Rodney, ~700km north (Fig. 1.2).

1.4. Statistical analysis

The computer programme "Number Cruncher Statistical Systems 97" developed by Dr JL Hintze (Utah, USA) was used for all statistical analyses in this thesis.

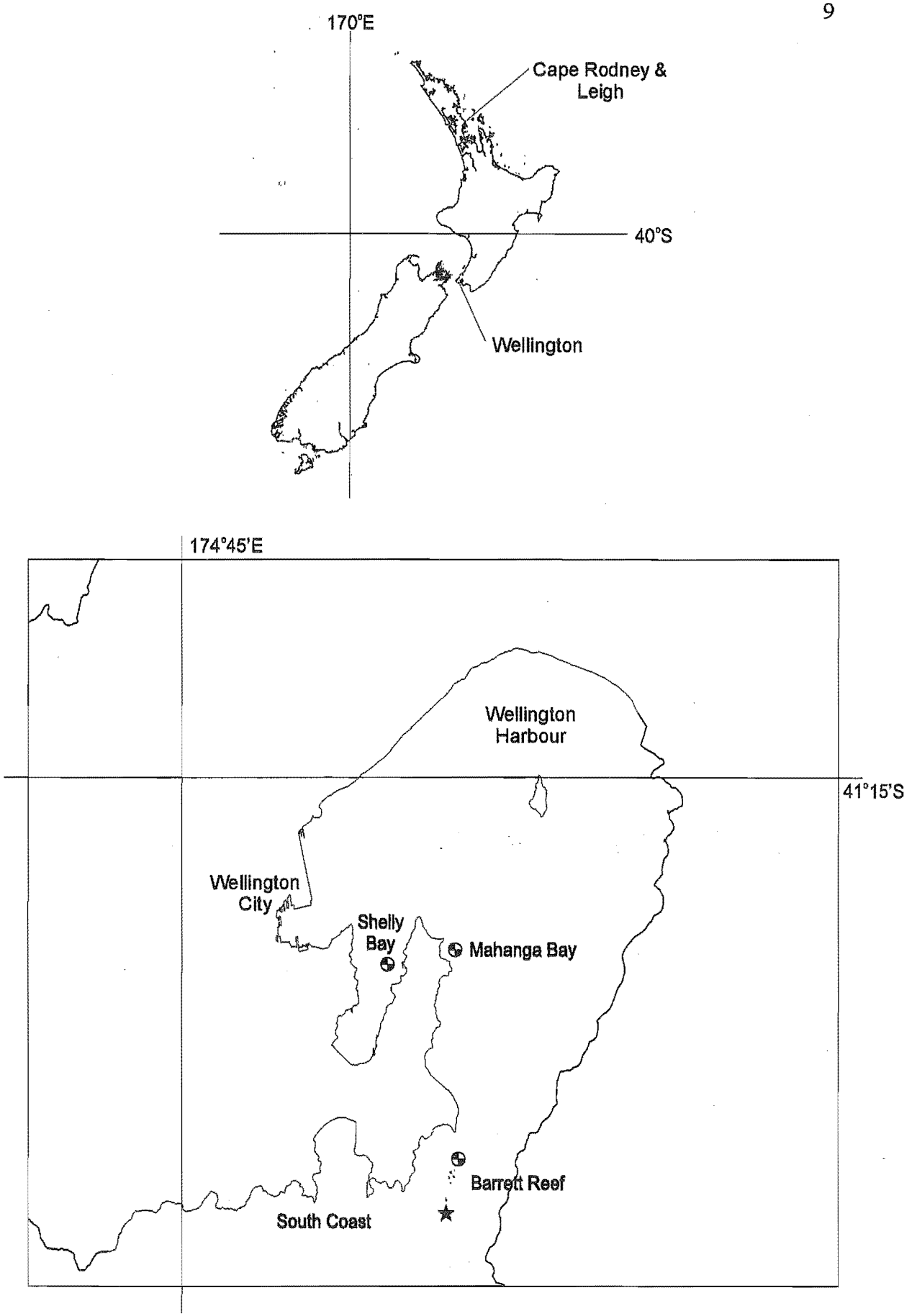


Figure 1.2. Map of New Zealand showing the locations of Wellington and Cape Rodney / Leigh. Enlarged map of Wellington Harbour shows the farming sites ◔ and the ecological monitoring site ★.

Chapter 2. Ecology of *Latrunculia brevis* and *Polymastia croceus*

2.1. Introduction

Sponges are an important component in many reef habitats in terms of biomass and diversity (Reiswig 1973, Dayton et al. 1974, Bergquist 1978, Schmahl 1990, Schubauer et al. 1990) and they may interact with the wider community in several important ways. Sponges can provide food (Ayling 1981, Wulff 1994, Pawlik 1998) or shelter (Costello and Myers 1987, Duffy 1992) for other organisms. They can also filter and extract much of the available phytoplankton (Reiswig 1971a, Pile et al. 1996, 1997, Bell et al. 1999) to the possible detriment to other suspension feeding organisms. Sponges can compete for and dominate the substrate (Dayton et al. 1974, Vincete 1978, Barthel 1988) and thereby exclude other organisms from settling and recruiting into the community.

Although sponges, as a phylogenetic group, are significant community determinators, their ecology has been examined in relatively few species (e.g., Stone 1970, Dayton et al. 1974, Ayling 1980, Battershill and Bergquist 1990, Turon et al. 1996). Sponge growth and biosynthesis of bioactive metabolites, in particular, are poorly understood. Bioactive metabolites, it has been suggested, aid in competitive interactions or in preventing predation or surface overgrowth of fouling organisms (Bakus et al. 1986, Hay 1996). Because their synthesis represents a drain on resources which could otherwise be channelled into growth or reproduction, sponges may only synthesise metabolites when they are most required (Turon et al. 1996), and this may lead to seasonal patterns of bioactivity (Green et al. 1990, Turon et al. 1996, Swearingen and Pawlik 1998). Some sponges also show seasonal variation in growth (Simpson 1968, Elvin 1976, Fell and Lewandrowski 1981, Barthel 1986, Turon et al. 1998) possibly influenced by seasonal cycles of water temperature, but this temporal pattern is not found in all species (Ayling 1983, Hoppe 1988, Pansini and Pronzato 1990). The population dynamics of a species encompasses such aspects as recruitment and mortality (Kingsford and Battershill 1998). Recruitment to the population is dictated by reproductive activity, which involves sexual or asexual processes in sponges (Bergquist 1978, Ayling 1980, Simpson 1984), and post-settlement mortality. Mortality of juvenile and adult sponges may result from

environmental events such as burial by sediment (Bakus 1968, Reiswig 1973) or biological interactions such as predation (Dayton et al. 1974, Ayling 1981).

These studies indicate that season and inter-annual events may affect the growth, survival, recruitment and metabolite biosynthesis of sponge species. Therefore, longer-term studies are required to understand sponge ecology. In this study, the population dynamics, growth and levels of metabolite biosynthesis of *Latrunculia brevis* and *Polymastia croceus* were examined on an exposed subtidal reef in Wellington over several years. Permanent or fixed quadrats were used because growth and survival of known sponges could be monitored through time (Creese and Kingsford 1998). *P. croceus* is common subtidally throughout New Zealand, and some aspects of its ecology, particularly its reproductive biology, have been studied in northern populations (Ayling 1980, Battershill and Bergquist 1990). These studies have discovered that *P. croceus* is able to reproduce both sexually and asexually and that reproductive activity varies between seasons. There is no published information on the ecology of *L. brevis*. Production of biologically-active metabolites was examined by measuring activity against the P388 murine leukaemia bioassay. This bioassay is a useful indicator for examining general levels of bioactivity in extracts from organisms as a preliminary screen (Blunt et al. 1990). The P388 assay obviously has no ecological meaning for the two species examined but because metabolites may have several ecological roles (Becerro et al. 1997a) it is appropriate to use an assay which tests bioactivity on a general scale (Turon et al. 1996).

To develop farming procedures or to examine the farming potential of a species requires knowledge of its ecology (Kinne 1977). For example, to determine whether a farming method promotes growth of a sponge requires knowledge of its natural growth. Therefore, this ecological study will also provide the basic information to examine the influence of the farming environment and culture method on the growth, survival and bioactivity of farmed *L. brevis* and *P. croceus*. Ultimately, this will lead to development of methods and procedures to farm sponges successfully at commercial scales.

2.2 Methods

2.2.1. Experimental layout

At the southern, exposed end of Barrett Reef (Fig. 1.2), where both *Latrunculia brevis* and *Polymastia croceus* are common, two $4m^2$ fixed quadrats were set up to monitor sponge growth, survival and recruitment for each species. Quadrats $4m^2$ in size

were chosen because they are known to be of sufficient size to provide meaningful data on the dynamics of sponge populations (Kingsford and Battershill 1998) and have been used in previous studies (e.g., Battershill 1986). In addition, they could all be monitored within one dive which was a prerequisite in this very exposed area. A tag was hammered into the rock to mark the 4 corners of each quadrat. The position of each quadrat was chosen to contain a high number of *L. brevis* (n = 25 and 24) or *P. croceus* (n = 9 and 8) across their size range. *L. brevis* is a more common species at Barrett Reef. The two *L. brevis* quadrats were 10m apart and situated at a depth of 12m on a large rock wall. The two *P. croceus* quadrats were 5m apart and situated at a depth of 14m on reef flat. The position of all *L. brevis* or *P. croceus* in each quadrat was mapped to monitor their individual growth.

2.2.2. Monitoring survival, recruitment and fusion

Generally, every 1-3 months each quadrat was monitored and sponge survival, recruitment, and fusion were recorded. Fusion occurs when two neighbouring sponges grow together and fuse into one sponge. A fusion event was easily identified *in situ* by comparing the size and position of all sponges against their previous positions mapped on a dive slate. An indentation in the ectosome marking where the two sponges fused could often be seen. A recruit was any new sponge that could be seen with the naked eye, approximately 5mm in diameter. This size has been used in other studies to examine the recruitment of sponges (e.g., Battershill and Bergquist 1990). Monitoring of *L. brevis* started in November `96 and finished in June `99. Monitoring of *P. croceus* started in August `97 and finished in June `99.

2.2.3. Measuring sponge size

A ruler was initially used to measure the height, width and length of each sponge. This method of size measuring is quick, nondestructive and has been used in other studies (e.g., Fell and Lewandroski 1981, Meroz and Ilan 1995). However, it was found to be unsuitable for measuring massive discrete sponges because of their complex morphologies. From May '97 onwards sponge volume was instead determined by the following method. First, each sponge was photographed next to a ruler to provide a scale and its height was measured to the nearest 0.5cm. A frame attached to the front of the camera and pressed against the rock adjacent to the sponge ensured the exact orientation of each photo. Next, the outline of each sponge was traced onto acetate (transparent) sheets. The trace was

then digitised and the graphics programme OPTIMAS used to calculate its basal area (cm^2). Basal area and height were then multiplied to calculate the volume of each sponge.

Specimens of *P. croceus* can greatly inflate in volume, possibly when feeding (Bell et al. 1999). This was easily identified by sponges having long extended papillae, fully open oscules and a "swollen" appearance. To remove this behaviour as a source of error, 5 *P. croceus* which were inflated one day and deflated the next were photographed and their volumes were compared between the two days. On average, deflated sponges were 0.63 (SE=0.09) the size of when they were inflated. Hence, the volume of any *P. croceus* photographed inflated was multiplied by 0.63 to give a better estimate of its volume.

At each monitoring the volume (cm^3) of 19 *L. brevis* and 13 *P. croceus* individuals found in the fixed quadrats was measured. Sponges which covered the size range of each species were chosen at the start of the experiment. For *L. brevis*, the initial volume of monitored sponges ranged from 2-344cm^3, while for *P. croceus*, the initial volume of sponges ranged from 4-440cm^3.

2.2.4. Encroaching organisms and bare space

Using photographs taken in June `98 (winter) and January `99 (summer) it was possible to determine what macro-organisms encroached onto *L. brevis* and *P. croceus*. This involved placing an acetate sheet with a grid pattern divided into 1cm^2 squares over each photo and counting each organism ≤2cm from the sponge. In addition, the percentage area of bare space around each sponge was determined in both winter and summer.

2.2.5. Monitoring bioactivity

Every few months a small amount of tissue was removed from 5 individuals of *L. brevis* and *P. croceus* located outside the four quadrats. Samples were stored in labelled bags and frozen at -20°C. At the end of the experiment, 2g of each sample was sent to the Chemistry Department at the University of Canterbury to be analysed against a P388 murine leukaemia screen. Each 2g sample consisted of 0.4g sub-samples cut from the 5 sponge pieces of each species. Each 0.4g sub-sample included ectosome and choanosome tissue. The bioactivity may vary between the ectosome and choanosome in sponges (Becerro et al. 1997b). Therefore, both sponge regions must be sampled and analysed to determine the overall pattern of bioactivity.

For chemical analysis, each 2g sample of *L. brevis* and *P. croceus* was dissolved in 20ml of MeOH or water, respectively, and then a two-fold dilution series of each sample was incubated for 72 hours with P388 cells (Gill Ellis, Chemistry Department, personal communication). The bioactivity of a sample is expressed as an IC_{50}, in ng/ml, and is the concentration of the sample required to reduce the P388 cell growth by 50% compared to control cells. The lower the IC_{50}, the more active the sample. An IC_{50} score below 1500ng/ml is considered very active (Lill et al. 1995).

The bioactivity of a sponge may depend on its size (Becerro et al. 1997b). This possible source of variation in bioactivity was eliminated in this study by sampling and analysing only sponges of a similar volume, approximately the size of a tennis ball. Both *L. brevis* and *P. croceus* were also sufficiently common around the quadrats that it was unlikely that the same individual was sampled twice, as repeat harvesting may have affected bioactivity. In addition, sponges were sourced from a large area at each monitoring to reduce the possibility of sampling from clones generated through asexual reproduction.

2.2.6. Statistical analysis

The ecology of *L. brevis* and *P. croceus* over the study period was analysed in the following way. The population dynamics of each species was separated into individual graphs showing mean percentage survival of original sponges, recruitment and fusion. The effect of sponge size on growth was examined by comparing the mean monthly growth rate of each photographed sponge over its initial volume in each season*year cell. The Spearman rank correlation coefficient determined whether initial sponge size influenced growth. The mean monthly growth rate was determined by the formula:

$$\text{growth rate} = ((\text{volume}_m - \text{volume}_{m-1}) / \text{volume}_{m-1}) / n \text{ month}$$

where volume_m and volume_{m-1} are the volume of a sponge at monitoring m and at the previous monitoring, respectively, and n month is the number of months between the two monitoring events. This formula is adapted from Turon et al. (1998). Because several months may separate consecutive monitorings the growth rate over each period is slightly skewed but it was sufficient to examine any possible differences between seasons and

sponge sizes. Because sponge size did not affect the growth rate of either *L. brevis* (Fig. 2.2) or *P. croceus* (Fig. 2.6), the average growth, expressed as percentage of initial volume, of each species was graphed over time. Only sponges which survived to the end of the experiment were used. Finally the bioactivity of each species over the study period was shown. The water temperature over the study period is included on all graphs to indicate any possible seasonal relationships with the population dynamics, growth and bioactivity of *L. brevis* and *P. croceus*. The temperature was recorded daily at a depth of 5m by the NIWA Mahanga Bay Hatchery. The water temperature was greatest in summer (20°C) and lowest in winter (9°C).

2.3. Results

2.3.1. *Latrunculia brevis*

Overall mortality of original sponges over the study period was low and similar between the seasons for each quadrat (Fig. 2.1a). No signs of predation, disease or aggressive competitive interactions with neighbouring organisms were observed that could explain why some *L. brevis* died. Two sponges were observed to become progressively smaller over several months until they eventually disappeared. The spongivores *Parika scaber* (teleost) (Ayling 1981, Battershill and Bergquist 1990) and *Aphelodoris luctuosa* (opisthobranch) were seen on several occasions in the study area. Recruitment occurred in all seasons but varied between quadrats (Fig. 2.1b), indicating variation over small spatial scales. Most recruits were found further than 10cm away from a conspecific. Apart from 3 recruits that budded off 2 sponges in August `97, it was not possible to determine without genetic analysis whether a recruit was the product of sexual or asexual reproduction. After 6 months, all 3 recruits had fused with their larger neighbour to reconstitute a single sponge (Fig. 2.1c). Excluding these 3 buds, 6 of the 13 sponges that recruited into the study quadrats during the monitoring had died by June `99. Both recruitment and mortality may possibly be under-reported because sponges that died shortly after recruiting may have escaped detection.

In each season*year cell, the initial size of a sponge did not affect its growth rate (Fig. 2.2). Growth rates varied greatly between sponges in each season*year, with some shrinking while others grew (Fig. 2.2). Only 14 out of the original 19 *L. brevis* photographed in May `97 survived to the end of the study. Of these 14 sponges only 1 sponge was larger after two and half years. This sponge grew from 87cm$^3$ in May `97 to be 140cm^3 in June `99. The other 13 sponges had shrunk to about half their original size by June `99 (Fig. 2.3). Sponges mostly shrank as water temperature fell (Fig. 2.3). Sponges grew as water temperature rose in `97 but not in `98.

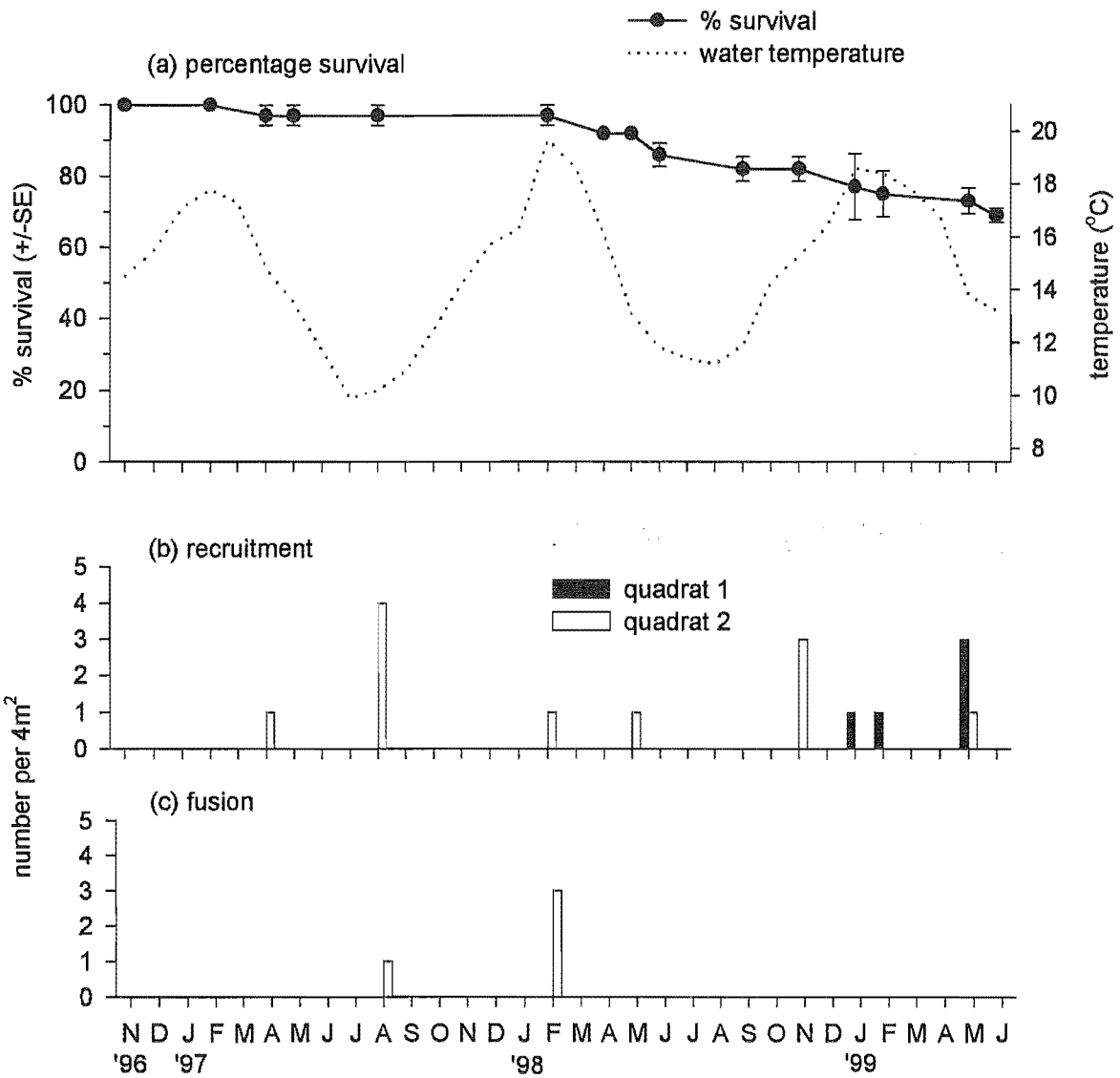


Figure 2.1. Population dynamics of *L. brevis* in Wellington from November '96 to June '99, showing mean percentage survival of original sponges, recruitment and fusion events. Error bars in percentage survival represent variation between quadrats. Recruitment and fusion both separated into quadrats. The water temperature is also shown.

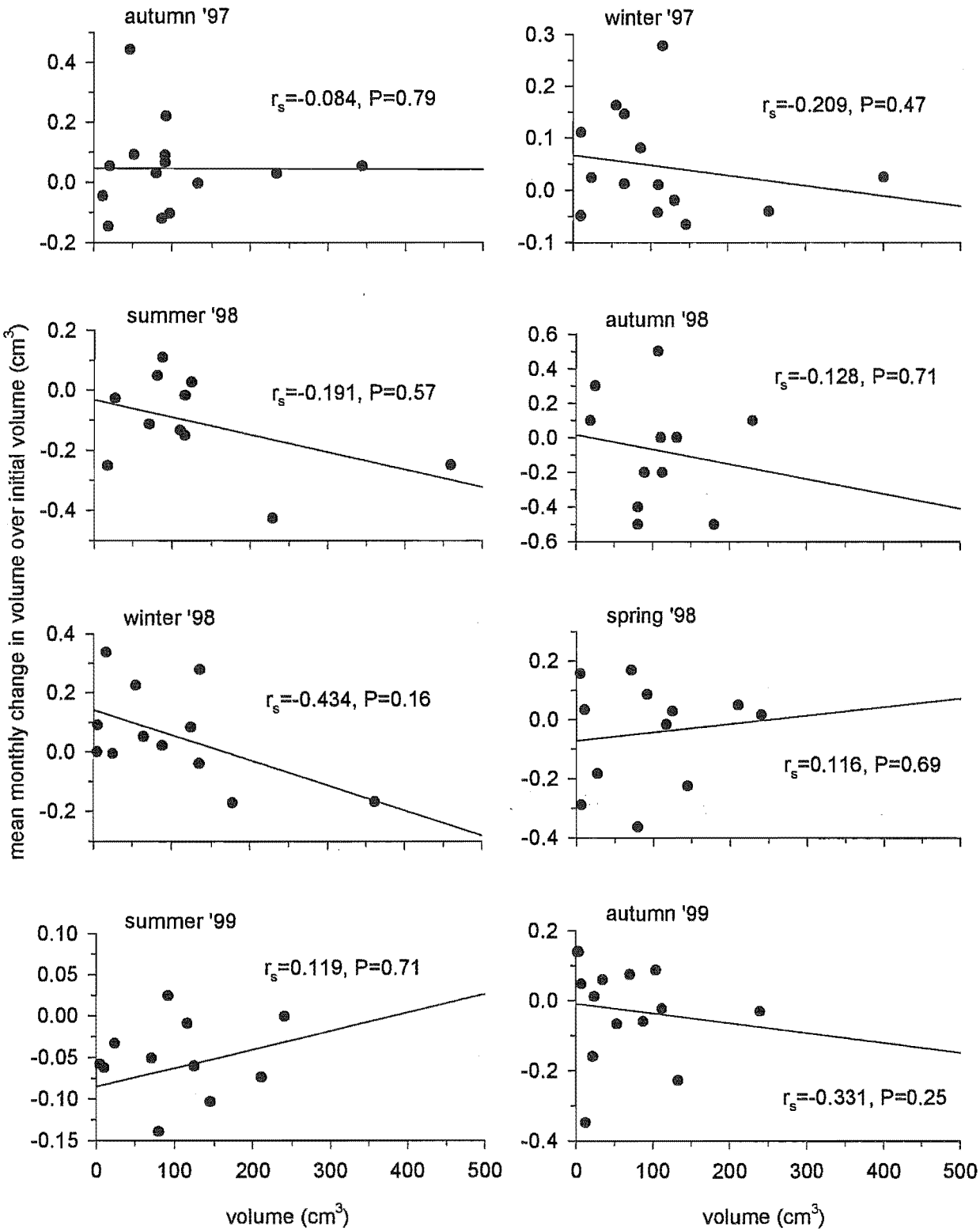


Figure 2.2. Comparison of growth rate to sponge volume for *L. brevis* in each monitored season*year cell. No data for spring '97. For each season*year cell, the Spearman rank correlation coefficient (r_s) between growth rate and sponge volume is shown.

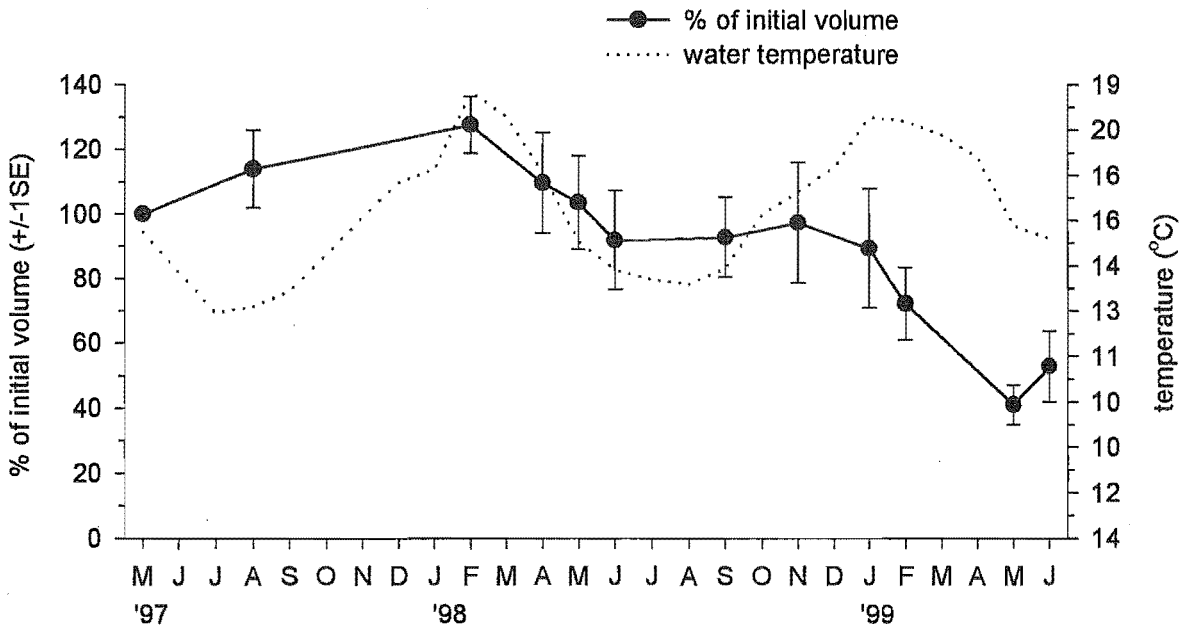


Figure 2.3. Mean percentage of initial volume of *L. brevis* from May '97 to June '99. The water temperature is also shown.

The most common macro-organisms encroaching *L. brevis* were the ascidian *Didemnum candidum*, red algae and bushy bryozoans of the genera *Costaticella*, *Orthoscuticella* and *Emma* (Table 2.1). More *L. brevis* were encroached by these organisms in summer. This meant that there was only a small percentage of bare or unoccupied space around sponges, particularly in summer (Table 2.1). *Didemnum candidum* was an occasional fouling organism on *L. brevis*, covering up to 20% of a sponge. An unidentified small tufting red alga also fouled some sponges.

Table 2.1. Mean percentage bare space (±1SE) and common encroaching organisms ≤2cm from *L. brevis* (n=12) in winter (June `98) and summer (January `99). The numbers alongside each encroaching organism represents the percent of the *L. brevis* population in each season encroached by a particular organism. Bushy bryozoans represent species of the genera *Costaticella*, *Orthoscuticella* and *Emma*.

	winter (%)	summer (%)
Bare space	17 (2)	7 (2)
Sponges		
Ancorina alata	8	8
Chondropsis sp.	25	25
Cliona celata	17	17
Polymastia massilis	17	17
Tethya ingalli	8	8
Ascidians		
Botryloides sp.	8	8
Cnemidocarpa bicornuta	25	25
Didemnum candidum	67	83
Bryozoans		
bushy bryozoans	58	75
Algae		
Corallina officinalis	17	17
red algae	83	92

The bioactivity of *L. brevis* extracts varied between seasons. Generally, bioactivity was high (low IC_{50}) during spring and low during summer and autumn (Fig. 2.4). However, bioactivity in summer varied greatly between years, being comparatively low (IC_{50}=1800) in March `97 and high ($IC_{50}$=350) in February `99.

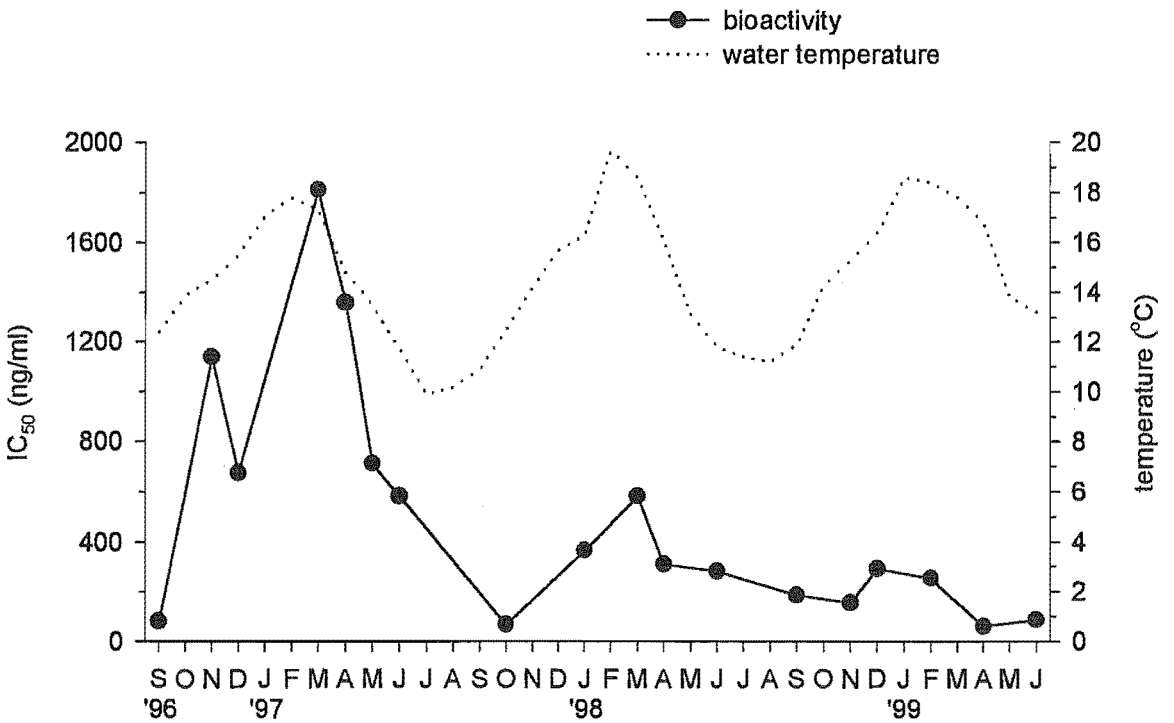


Figure 2.4. Bioactivity (IC_{50}) of *L. brevis* from September '96 to June '99. Each dot represents the bioactivity of a pooled sample from 5 sponges. The water temperature is also shown. As IC_{50} decreases, bioactivity increases. An IC_{50} <1500 is considered very active.

2.3.2. *Polymastia croceus*

Mortality of original sponges was low over the study period and similar between the seasons (Fig. 2.5a). As for *L. brevis*, no signs of predation, disease or aggressive competitive interactions with neighbouring organisms were observed that could explain why some *P. croceus* died. Recruitment occurred mostly in autumn (Fig. 2.5b), and resulted from either sexual or asexual reproduction involving the production of larvae, or the disintegration of a sponge producing buds. The latter process was observed once and produced 10 buds over a 2 month period. Only 3 buds survived to June '99. Most recruits (22/25) were found less than 10cm away from an existing sponge or where the sponge was

before it broke up. Initial mortality of recruits was high; 14 of the 25 recruits dying within a few months of settling. Fusion was recorded only once over the study period (Fig. 2.5c).

In each season*year cell, the initial size of a sponge did not affect its growth rate (Fig. 2.6). Like *L. brevis*, growth rates of *P. croceus* varied greatly between sponges in each season*year, with some sponges shrinking while others grew (Fig. 2.6). Growth of *P. croceus* showed a seasonal pattern, with sponges generally growing during winter and spring as the water temperature rose and shrinking during summer and autumn as the water temperature fell (Fig. 2.7). After two years, *P. croceus* had on average doubled in size.

The most common macro-organisms encroaching onto *P. croceus* in winter and summer were *Caulerpa brownii*, red algae and bushy bryozoans of the genera *Costaticella*, *Orthoscuticella* and *Emma* (Table 2.2). In both winter and summer, ≥50% of the immediate area around each sponge was bare space. No fouling organisms were observed on any *P. croceus* individual.

An examination of the inflation/deflation patterns of *P. croceus* photographed at each monitoring indicated a relationship with water clarity. When the surrounding water was very turbid and full of suspended particles (visibility <2m) sponges were deflated and had closed their oscules, indicating that there was no pumping activity. However, in clear water (visibility >10m) sponges were inflated, had long extended papillae with open oscules, and appeared to be feeding. In normal conditions of water clarity in Wellington (visibility 2-10m) some sponges were inflated while the rest were deflated. Variability in inflation was also observed within an individual. On several occasions, one part of a sponge was observed inflated with its oscules open while the remainder of the sponge was observed deflated with its oscules closed. For growth analysis of these individuals, the volume of the inflated segment was determined separately, multiplied by 0.63, and then added to the volume of the deflated segment.

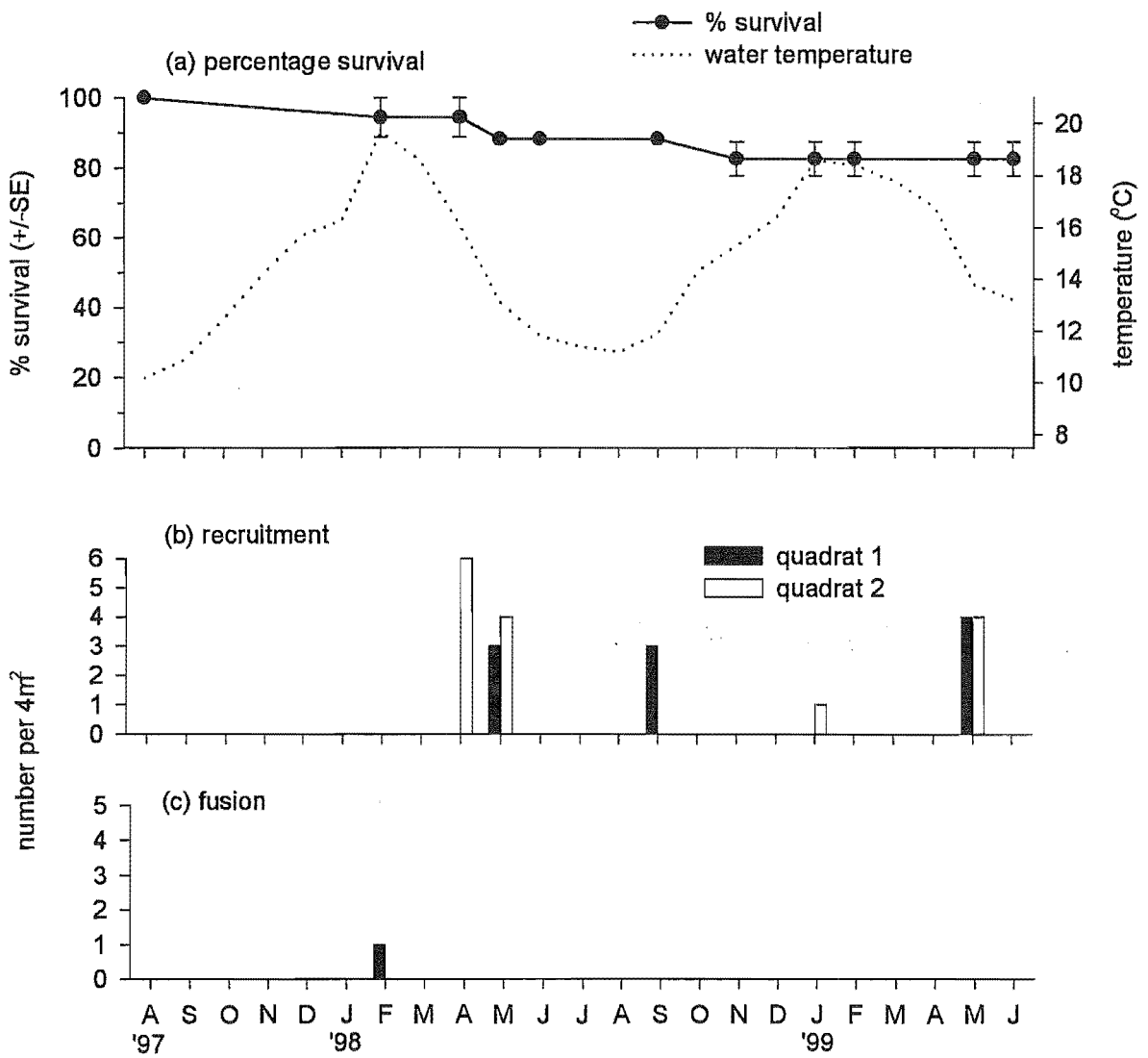


Figure 2.5. Population dynamics of *P. croceus* in Wellington from August '97 to June '99, showing mean percentage survival of original sponges, recruitment and fusion events. Error bars in percentage survival represent variation between quadrats. Recruitment and fusion both separated into quadrats. The water temperature is also shown.

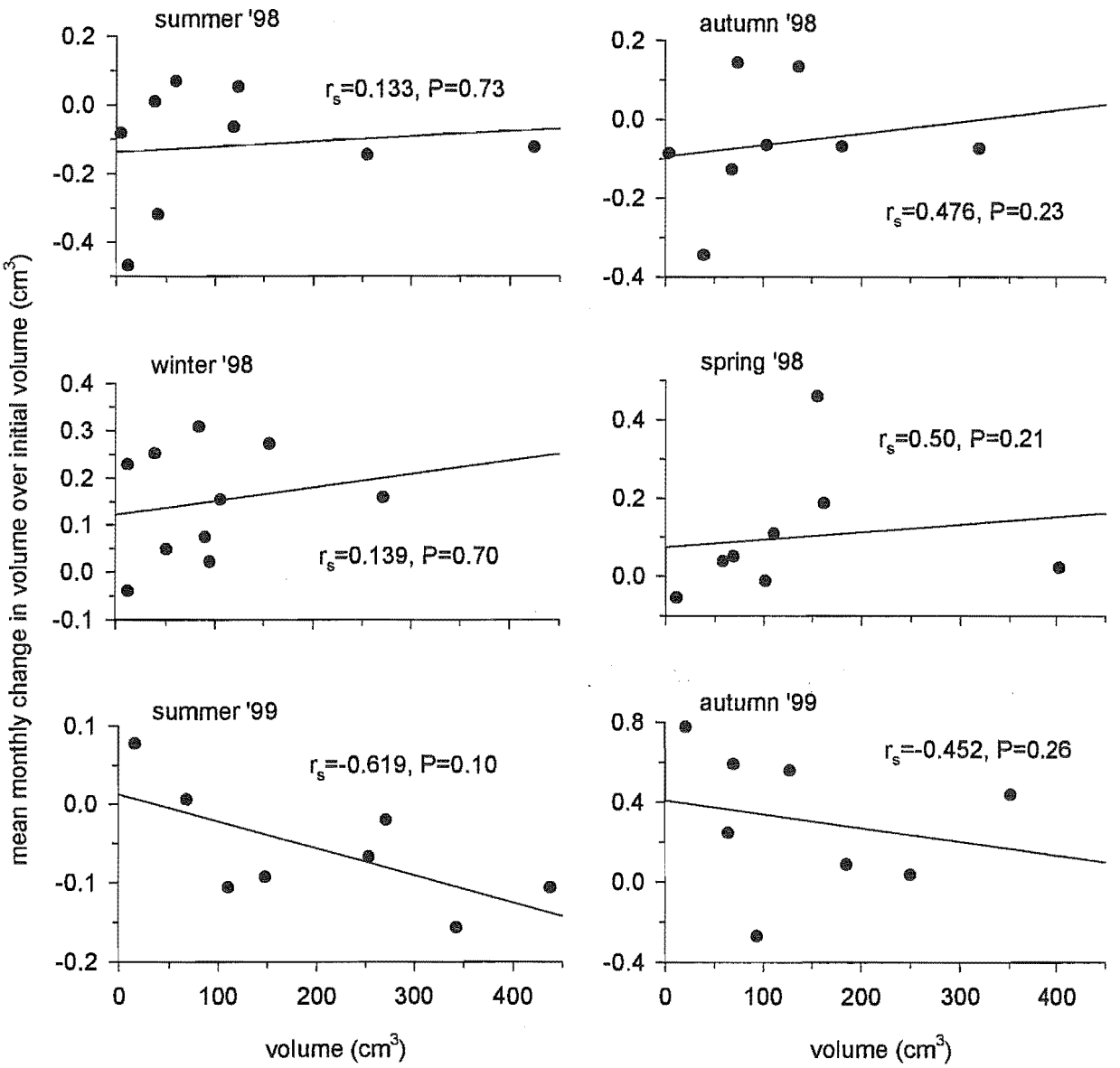


Figure 2.6. Comparison of growth rate to sponge volume for *P. croceus* in each monitored season*year cell. For each season*year cell, the Spearman rank correlation coefficient (r_s) between growth rate and sponge volume is shown.

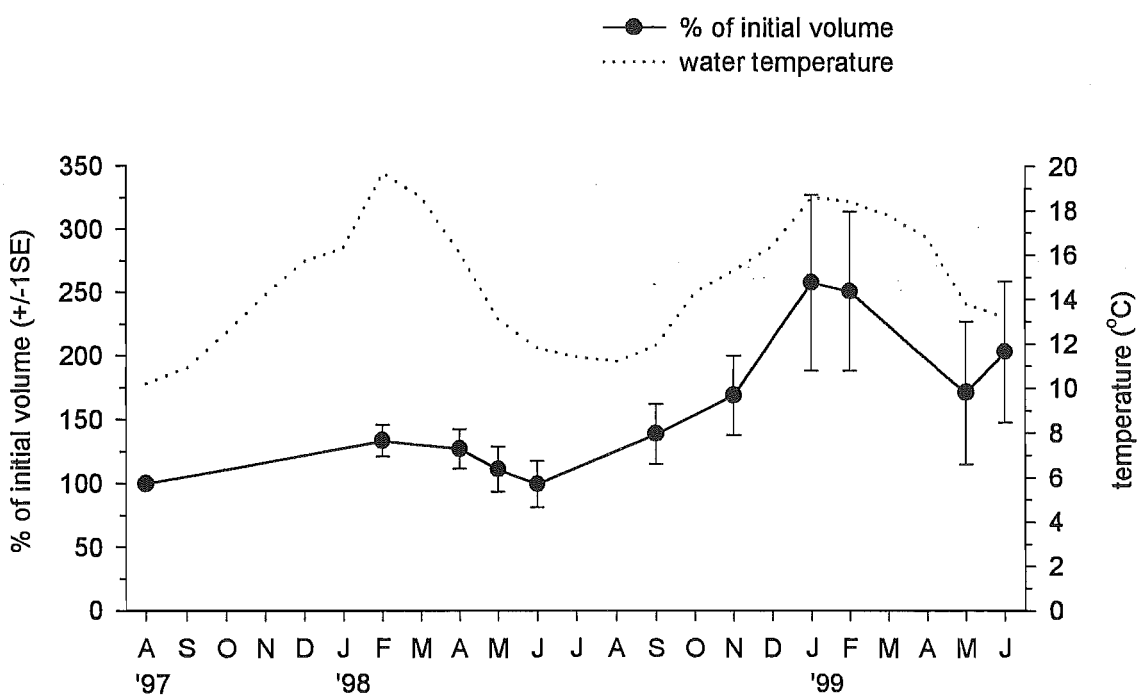


Figure 2.7. Mean percentage of initial volume of *P. croceus* from August '97 to June '99. The water temperature is also shown.

Table 2.2. Mean percentage bare space (±1SE) and common encroaching organisms ≤2cm from *P. croceus* (n=10) in winter (June `98) and summer (January `99). The numbers alongside each encroaching organism represents the percent of the *P. croceus* population in each season encroached by a particular organism. Bushy bryozoans represent species of the genera *Costaticella*, *Orthoscuticella* and *Emma*.

	Winter (%)	summer (%)
Bare space	66 (5)	51 (4)
Sponges		
Cliona celata	10	10
Sea anemones		
Actinothoe albocincta	30	30
Ascidians		
Cnemidocarpa bicornuta	20	20
Didemnum candidum	30	20
Bryozoans		
bushy bryozoans	40	60
Algae		
Caulerpa brownii	40	50
Corallina officinalis	30	30
red algae	70	80

The bioactivity of *P. croceus* extracts varied greatly over time, but showed no seasonal pattern (Fig. 2.8). *P. croceus* at each monitoring was either highly active (low IC_{50}) or very inactive.

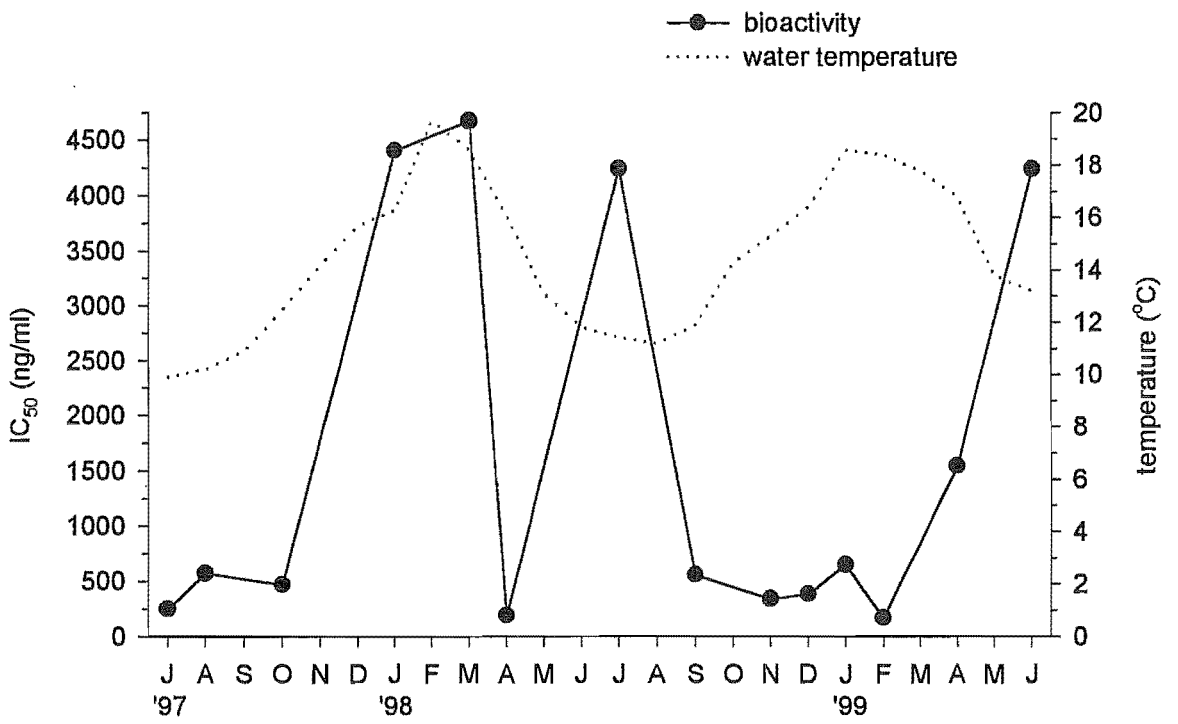


Figure 2.8. Bioactivity (IC_{50}) of *P. croceus* from July '97 to June '99. Each dot represents the bioactivity of a pooled sample from 5 sponges. The water temperature is also shown. As IC_{50} decreases, bioactivity increases. An IC_{50} <1500 is considered very active.

2.4. Discussion

As in many subtidal species (Reiswig 1973, Dayton 1979, Turon et al. 1998), the mortality of adult sponges of *Latrunculia brevis* and *Polymastia croceus* was low and appeared unaffected by seasonal cycles of water temperature. Recruitment patterns, however, varied greatly between the two species. For *L. brevis*, recruitment occurred in all seasons and varied between the two quadrats, indicating variation over small spatial scales. In contrast, recruitment of *P. croceus* in both quadrats occurred mostly in autumn. Autumn recruitment events concur with a study by Ayling (1980) who found that *P. croceus* in the far north of New Zealand produces sexual and asexual reproductives in summer and early autumn only. Ilan (1995), studying reproductive activity in the family Latrunculiidae, found that the Red Sea sponge *Negombata* (*Latrunculia*) *magnifica* produces sexual reproductives throughout the year except during winter. For *L. brevis*, recruitment occurred in all seasons, including winter when several buds were produced.

These results indicate that *L. brevis* is reproductively active, either sexually or asexually, throughout the year.

The pattern of recruitment also varied between the two species. Most recruits of *L. brevis* were found further than 10cm away from a conspecific, while recruits of *P. croceus* were generally found clumped and situated close (<10cm) to an established sponge. These differences in recruitment patterns may result from several factors, including rock gradient. Leys and Lauzon (1998) studied the hexactinellid sponge *Rhabdocalyptus dawsoni* on rock walls in Canadian fjords and found that few juveniles were near parent sponges because of the steep gradient of the rock. The *L. brevis* population examined in this study was also situated on a rock wall. Therefore, it is likely that the steep gradient promoted reproductives of *L. brevis* to settle at least 10cm away from the parent sponge. In contrast, the *P. croceus* population was situated on reef flat with no gradient. The amount of bare rock space around sponges also differed between the two species. For *L. brevis*, much of the surrounding substratum was occupied with encroaching organisms, particularly algae, ascidians and other sponges. This would restrict recruitment to areas of bare rock situated away from the parent sponge. *P. croceus*, in contrast, had fewer encroaching organisms so more of the surrounding area was available for the recruitment of conspecifics. Lastly, *P. croceus* often produces reproductives in large groups, either as an adhesive mass of 70 or more oocytes from sexual reproduction (Ayling 1980) or in a long "bead" formation containing many asexually derived buds (Battershill and Bergquist 1990). These reproductives move only short distances (~1cm) before they attach to the substratum (Battershill and Bergquist 1990). This will promote clumping of juveniles around the parent sponge.

Most recruits of *L. brevis* and *P. croceus* died a few months after recruiting into the populations. Poor survival of recruits is found in many sponge species (Reiswig 1973, Fell and Lewandrowski 1981, Meroz and Ilan 1995) and it can affect the distribution patterns of adult sponges (Battershill and Bergquist 1990). The factors that cause mortality of sponge recruits are mostly unknown, although several studies have discovered that urchins can kill juvenile sponges by grazing or abrasion (Ayling 1980, 1981, Maldonado and Uriz 1998). No urchins were however observed in the study area, they are rare in very exposed areas (Andrew 1988) such as the southern end of Barrett Reef where this study was done. The spongivores *Parika scaber* (Ayling 1981, Battershill and Bergquist 1990) and *Aphelodoris luctuosa* were occasionally seen in the study area and

may have removed some recruits. Alternatively, some recruits may have simply detached in storms and been swept away by currents.

Although predation may remove some recruits, it is unlikely that predation greatly affects the survival of adult *L. brevis* or *P. croceus*. A separate study examining the harvesting of wild populations as a method to supply bioactive metabolites determined that *L. brevis* and *P. croceus* take ~1 and ~3 months, respectively, to heal cut surfaces (Chapter 6). This indicates that if predation was common then some evidence such as bite marks would be observed during the study period. However, no bite marks were seen on any adult sponge. The presence of bioactive metabolites in *L. brevis* and *P. croceus* may prevent predation as noted for other sponge species (Nèeman et al. 1975, Green 1977, Bobzin and Faulkner 1992, Pawlik et al. 1995, Chanas et al. 1996,).

Bioactive metabolites can also be biosynthesised by encroaching organisms competing for space (Bakus et al. 1986, Proksch 1994, Hay 1996). It is possible, therefore, that the bioactive metabolites present in *L. brevis* and *P. croceus* are also used to aid in competition interactions with encroaching organisms. The metabolites may have also promoted the high incidence of static boundary interactions or stand-offs (Russ 1982) observed in this study. Because no *P. croceus* and few *L. brevis* were fouled with epibionts, their bioactive metabolites may also be used to prevent surface overgrowth of fouling organisms, as suggested for other sponge species (McCaffrey and Endean 1985, Thompson et al. 1985, Bakus et al. 1990, Davis et al. 1991). An occasional fouling organism on *L. brevis* was the ascidian *Didemnum candidum*. In allelochemical interactions, ascidians are generally considered competitively dominant over sponges (Kay and Keough 1981, Russ 1982).

This study suggests that bioactive metabolites present in *L. brevis* and *P. croceus* may have several ecological roles: to prevent predation, to aid in competitive interactions and to deter bio-fouling. Because the chemical analysis examined tissue portions and not specific metabolite extracts it is unknown whether this chemical defence is from one or several bioactive metabolites. Multiple ecological roles for specific sponge metabolites has been proposed for some time (McCaffrey and Endean 1985, Thompson 1985) but only recently have studies tested this using ecologically-relevant organisms. For example, bioassay tests using sea urchin eggs and bryozoan larvae indicate that the low polar extract (DCM fraction) from the sponge *Crambe crambe* can be used to aid in competitive interactions and to inhibit surface overgrowth of fouling organisms (Becerro et al. 1997b).

Preventing the settlement and surface overgrowth of fouling organisms may explain the seasonal patterns of bioactivity in *L. brevis*. Temperate organisms generally settle and recruit during spring (Underwood and Anderson 1994), and in this study, greatest recruitment of organisms, particularly algal species, was observed in spring. Therefore, *L. brevis* may have been more biologically active in spring to prevent the relatively high seasonal recruitment of marine organisms from settling and fouling its surface. A seasonal pattern of bioactivity has been reported in several other species. Turon et al. (1996) found that *Crambe crambe* is more biologically active in summer and autumn, and they suggested that seasonal patterns result from reproductive needs, diverting energy away from biosynthesis into reproductive activity, and from environmental factors, such as seasonal variation in competitors. Green et al. (1990) discovered that several species from Mexican waters have greater anti-microbial activity during the warmest season possibly to defend against bacterial disease which is more prevalent at high water temperatures. Swearingen and Pawlik (1998) found that *Chondrilla nucula* is more toxic to fish predators in summer.

In contrast to *L. brevis*, *P. croceus* showed no seasonal pattern in bioactivity, but exhibited two modes of bioactivity, being either active or very inactive. Since *P. croceus* was active at most sampling dates this mode probably represents the "normal" bioactivity. The inactive mode is unlikely to be an experimental artefact because the experimental design involved sampling 5 randomly chosen sponges, of similar size, collected from a large area. In addition, the 4 inactive samples all had similar IC_{50} scores indicating that all 5 sponges in each sample were inactive. The dates that *P. croceus* was inactive appeared random and did not correlate well with its known reproductive cycle. Thus, it is unlikely they result from a diversion of energy into reproductive activity as found for *Crambe crambe* (Turon et al. 1996). To fully understand what physiological or environmental factors could induce very low bioactivity in *P. croceus* it may be necessary to examine each bioactive metabolite individually. This was not possible in this study. It is interesting to note that *P. croceus* could change from being very inactive to being active in one month which indicates a rapid biosynthesis of bioactive metabolites.

Metabolite biosynthesis costs the organism an investment of energy (Whittaker and Feeny 1971, Becerro et al. 1997b). This cost is the basis of the optimal defence theory (Fagerström et al. 1987) which proposes that the organism will only produce a defense, such as bioactive metabolites, when it most required. The seasonal pattern of bioactivity in

L. brevis and the active/inactive response of *P. croceus* suggests that both species increase metabolite biosynthesis when the chemical defence is most required.

Growth of *L. brevis* and *P. croceus* varied greatly with season. For both species, individuals generally grew during winter and spring as the water temperature increased and shrank during summer and autumn as the water temperature decreased. Greatest growth as the water temperature rises has been noted for several sponge species. For example, Simpson (1968) discovered that *Microcinia prolifera* starts growing once the temperature rises above 10°C. *Halichondria* sp. grows as water temperature increases (Fell and Lewandrowski 1981). Turon et al. (1998) found that maximum growth of *Crambe crambe* coincides with the seasonal rise in water temperature. Barthel (1986) discovered that growth of *Halichondria panicea* is arrested even if food is abundant until there is a rise in water temperature. Therefore, water temperature can have a greater influence on growth than does food abundance for at least one species. Alternatively, some sponge species grow best when water temperature falls (Johnson 1979), others show no seasonal pattern in growth (Ayling 1983, Hoppe 1988, Pansini and Pronzato 1990).

Why growth patterns among sponges vary so greatly is unknown, although seasonal growth of some species may result from seasonal differences of food availability. Sponges feed primarily on ultraplankton (<10μm) (Reiswig 1971a, 1975, Van de Vyver et al. 1990, Pile et al. 1996, 1997, Bell et al. 1999) which generally increase in abundance as water temperature rises (Fogg 1986, Joint 1986, Waterbury et al. 1986, Tamigneaux et al. 1995). Falling food abundance probably accounts for many sponges shrinking after summer but for some species reproductive investment may also be a factor. Reproduction is generally considered a drain on resources which may deflect energy away from somatic growth (Sebens 1987), particularly so in sponges, where gametogenesis involves the transformation of choanocytes into sperm sex cells which reduces the overall feeding activity of a sponge (Simpson 1984). Both Elvin (1979) and Barthel (1986) studying *Haliclona permollis* and *Halichondria panicea*, respectively, suggested that adult sponges degenerate after reproduction in summer. *P. croceus* is reproductively active in summer and early autumn only (Ayling 1980) and in my study it mainly recruited in autumn. Therefore, its seasonal patterns of growth may also result from seasonal variation in reproductive investment. For some species, however, reproductive investment does not account for seasonal growth patterns. Turon et al. (1998) found that small sponges of *Crambe crambe* showing no reproductive activity also vary in size between seasons. In the

present study, recruitment of *L. brevis* occurred in all seasons suggesting that it is reproductively active throughout the year and, therefore, its seasonal growth patterns are unlikely to result from seasonal variation in reproductive investment.

For both *L. brevis* and *P. croceus*, growth varied greatly between the monitored sponges, irrespective of season, some sponges increased in size while others shrank. Variable growth is common to many sponge species (Storr 1964, Reiswig 1973, Dayton 1979, Fell and Lewandrowski 1981, Ayling 1983, Hoppe 1988, Wulff 1991, Leys and Lauzon 1998) and it may result from several factors. In several species, small sponges grow faster than large sponges (Reiswig 1973, Hoppe 1988, Wulff 1991, Leys and Lauzon 1998). Growth would therefore vary within a population consisting of sponges across the size range of a species. However, similar growth rates between small and large sponges for both *L. brevis* and *P. croceus* indicate that sponge size did not cause the observed variation in growth.

Differences in reproductive investment among species and individuals may also lead to variation in growth. Ayling (1980) examined the reproductive activity of 10 temperate species and discovered that only some individuals of each species were reproductively active at a particular time. This suggests that within a population some sponges may invest in reproduction at the expense of growth. This trade-off may also explain annual variation in growth.

One interesting observation of this study was that sponges of *P. croceus* could contract their tissue and close their oscules when the surrounding water is very turbid and laden with sediment, and thus prevent their canals becoming blocked. The contraction of tissue or a reduction in pumping activity resulting from a high sediment load has been observed in many other sponge species (Reiswig 1971b, Wilkinson 1978, Gerrodette and Flechsig 1979). However, this behavioural response to sediment is not found in all species (Wilkinson 1978, Pansini and Pronzato 1990) and was not observed in *L. brevis*.

In summary, this study has discovered that adult mortality of *L. brevis* and *P. croceus* in Wellington is low and similar between the seasons. However, juvenile sponges had high mortality, possibly due to urchin grazing or being dislodged by storms. The bioactive metabolites present in *L. brevis* and *P. croceus* may have several ecological roles: to aid in competitive interactions and to prevent predation and surface overgrowth of fouling organisms. For both species, growth rates varied greatly between individuals and were unaffected by sponge size within the range examined. In general, both species

grow in winter and spring when the water temperature rises and shrink in summer and autumn when the water temperature falls.

Chapter 3. How the environment affects sponge farming

3.1. Introduction

To successfully farm sponges for metabolite production it is essential to determine how environmental factors such as season, exposure and depth, influence explant growth, survival and biosynthesis.

Only a few studies have farmed sponges experimentally to examine the effect of the environment on explant growth and survival. Verdenal and Vacelet (1990) farmed three *Spongia* species at 6 sites positioned from 0.3-50km away from a sewer outlet in the Mediterranean Sea, and discovered that explant growth and survival were both reduced in heavily-polluted water. They suggested that this was caused by the high sediment-load smothering the explants. They also found that growth and survival varied greatly between the three species, possibly because of differences in their ability to reorganise their tissue after cutting. Duckworth et al. (1997) farmed three New Zealand sponges, *Psammocinia hawere*, *Raspailia agminata* and *Raspailia topsenti*, at different depths in two sites differing in their degree of exposure. They discovered that explant growth and survival are generally poorer in shallow depths (5m) and at high exposure, probably due to the damaging effects of high light levels and strong water movement. They also found that *P. hawere* grew and survived better when transplanted in winter than in summer, possibly because of lower water temperature in winter reducing explant stress during transplanting. Contini (1995) discovered that growth of *Negombata (Latrunculia) magnifica* was greater at depths of 10m and 17m than at 7m.

Several transplant studies provide additional information on environmental effects on explant growth and survival. Moore (1908a) observed that explants of bath sponges transplanted in winter survive better than explants transplanted in summer. Wilkinson and Vacelet (1979) transplanted explants of 6 Mediterranean sponges species to different conditions of exposure and illumination and discovered that explant growth generally increases as exposure increases and that species which harbour symbiotic cyanobacteria grow best in illuminated conditions. Maldonado and Young (1998) found that two Caribbean sponges which naturally occur at depths above 40m can survive at depths of 100m but not at 300m. They suggested that the relatively cool water temperature of 19°C at 300m was responsible for the sponge mortality.

The environmental effect on metabolite biosynthesis of transplanted sponges has also been examined in only a few studies. Thompson et al. (1987) discovered that genetically identical explants of *Rhopaloeides odorabile* produce more diterpenes (bioactive metabolites) when transplanted and exposed to intense light, possibly to inhibit surface overgrowth of fouling organisms. Kreuter et al. (1992) discovered that if explants of *Aplysina* (*Verongia*) *aerophoba* collected from a depth of 30m are grown in illuminated conditions in the laboratory they biosynthesise a bioactive metabolite present only in individuals found above a depth of 10m. They suggested that UV light is required for the biosynthesis of the metabolite. In addition, several ecological studies have discovered that metabolite biosynthesis in sponges may vary with season, generally greatest production being in summer (Turon et al. 1996, Swearingen and Pawlik 1998).

These studies indicate that the environment will greatly influence the response of sponges in farming situations. However, they do not provide sufficient knowledge to farm sponges for metabolite production with confidence. To establish basic principles to farm sponges a study examining how season, exposure and depth affect explant growth, survival and biosynthesis of biomedically important sponge species is required.

To examine the effect of the farming environment on the commercial aquaculture of sponges two separate experiments were done. In the first experiment, *Latrunculia brevis* and *Polymastia croceus* were transplanted and farmed in each season at different exposures and depths. To examine the effect of each season restricted the farming period in each case to two months to ensure that growth would not run into the next season. Biosynthetic production of biologically-active metabolites was examined by measuring bioactivity against the P388 murine leukaemia bioassay. The P388 bioassay will not generate production values of the target metabolite, but it will indicate what effect an environmental factor, such as season, has on the biosynthesis of *L. brevis* and *P. croceus*. The second experiment involved a reciprocal transplant where *P. croceus* from two locations, ~700km apart, were transplanted and farmed in each location. Palumbi (1986) discovered that sponges grow and adapt to their local environment, so the reciprocal transplant experiment will examine whether farming explants in new locations influence their growth and survival. This will determine whether biomedically important sponges such as *P. croceus* can be transplanted and farmed in locations which are more suitable for commercial production.

3.2. Methods

3.2.1. Farming *Latrunculia brevis* and *Polymastia croceus* in different seasons, exposures and depths

3.2.1.1. Collecting and cutting sponges

At the start of each season approximately forty *L. brevis* and forty *P. croceus* were collected from the south coast of Wellington. To minimise harvesting impact one third of each sponge was left still attached to rock; monitoring showed that these cut sponges healed quickly.

All collected sponges were kept in tanks with running seawater at ambient temperature sourced from Wellington Harbour, and transplanted as soon as possible, normally within two days. *L. brevis* and *P. croceus* were separated into different tanks (50l) to prevent any antagonistic interactions between the two species. Sponges were cut with scalpels under running seawater into cube-shaped explants, approximately $16cm^3$ (2.5x2.5x2.5cm) in size. $16cm^3$ explants were of sufficient size not to fall through the mesh of the scallop lanterns, yet allowed high explant production from the 40 harvested sponges. All explants had at least one side uncut and covered with pinacoderm. Sterile surgical gloves were worn when handling sponges and explants to prevent contaminating them. Explants were randomised to treatments thus blocking across differences in initial condition.

3.2.1.2. Farming layout

Each season explants of *L. brevis* and *P. croceus* were transplanted and farmed in three exposures (Fig. 3.1), differing in the degree of water movement: high exposure (Barrett Reef) at the entrance to Wellington Harbour; moderate exposure (Mahanga Bay) and low exposure (Shelly Bay) within the harbour (Fig. 1.2). At the high and low exposure, sponges were farmed at depths of 5, 10 and 15m. The moderate exposure site allowed farming at depths of 5 and 10m only. The experimental design (Fig. 3.1) is therefore not orthogonal. However, because these sponges can grow naturally in deeper waters it was considered important to farm experimentally in deeper waters (15m) where that was possible. For all statistical analyses, unless otherwise stated, only the 5m and 10m depths were tested in full ANOVA models. Dive surveys determined that neither species is found naturally at the two inner harbour locations, nor at depths <7m in the outer harbour.

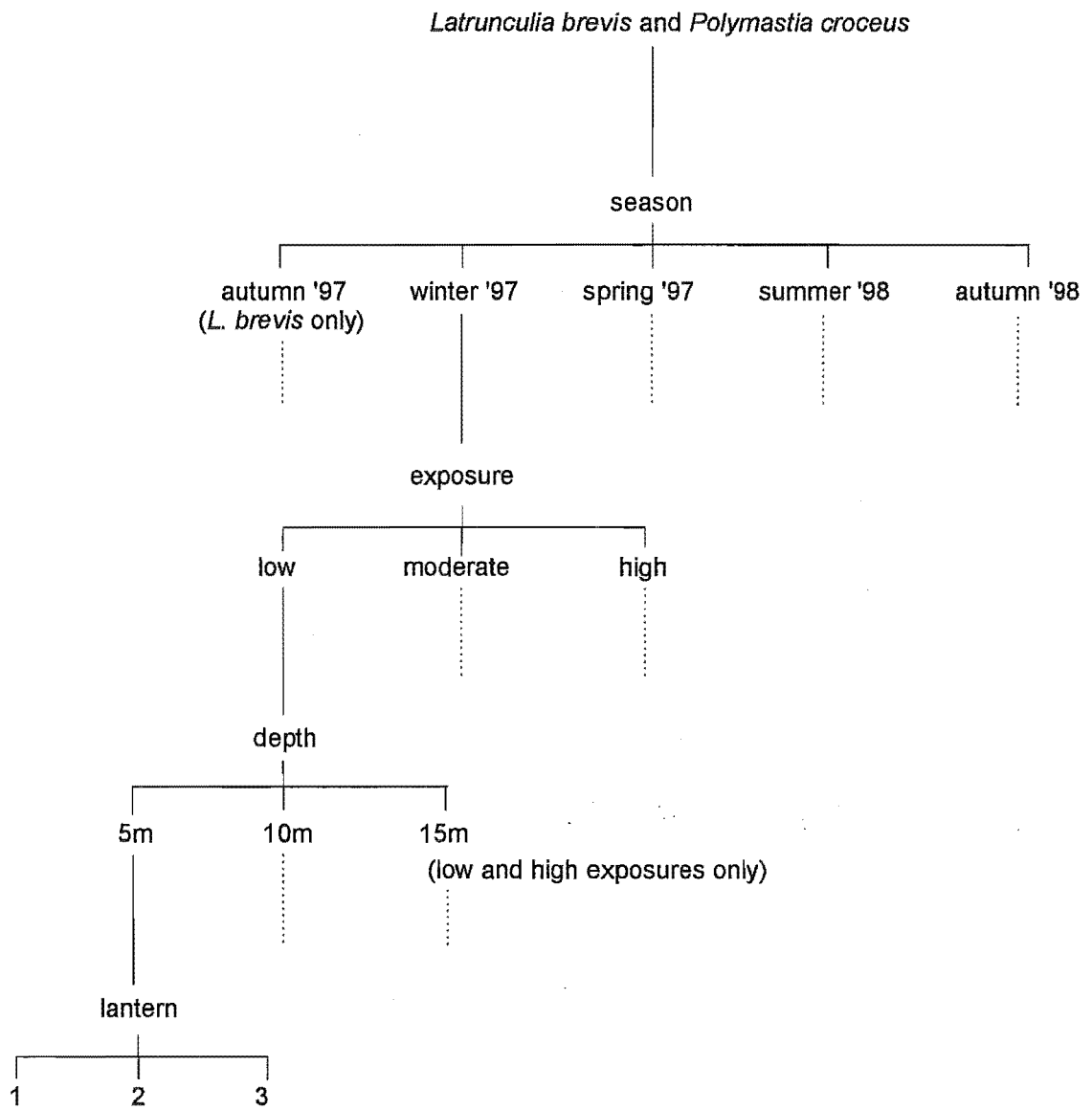


Figure 3.1. Experimental design for farming *L. brevis* and *P. croceus* between seasons, exposures and depths. The design is orthogonal except where noted. Dotted lines indicate where the design is mirrored.

Three culture arrays approximately 15m apart were established at each exposure. An array consisted of one scallop lantern at each depth linked by rope pulled taut by a subsurface buoy and anchored with heavy weights to the substrate (Fig. 3.2). A scallop lantern is a 1.5x.5m cylindrical net divided into ten compartments and covered with large mesh, with a percent mesh cover of 10% (i.e. 90% of the lantern is open to the flow of water). Scallop lanterns have been used successfully in earlier trials to grow the biomedically important sponge *Lissodendoryx* n. sp. (Battershill and Page 1996). A preliminary experiment determined that neither growth (One-Way ANOVA: $F_{df(9,71)}=0.56$, $P=0.83$) nor survival (One-Way ANOVA: $F_{df(9,8)}=0.73$, $P=0.68$) of explants is affected by which compartment they are farmed in within a lantern. For each species, 8 explants were placed all together into a randomly chosen and separate compartment in each scallop lantern. Each season, 192 explants of each species were farmed for two months. This ensured that farming would not run into the next season.

To gain a greater understanding of possible inter-annual variation in explant growth, survival and bioactivity, one species (*L. brevis*) was transplanted and farmed in autumn '97 and autumn '98.

3.2.1.3. Monitoring growth and survival

Growth was determined by wet-weighing the explants (to 0.1g) at the start and at the end of each experiment. Wet-weighing can give inaccurate growth results because sponges often contain a high proportion of water (Elvin 1979, Stone 1970). However, it was found that explants of *L. brevis* and *P. croceus* expel most excess water if they are disturbed and then left for 30mins (Fig. 3.3). Therefore, to obtain reliable measures of weight, explants were disturbed by handling and then waiting 30 minutes before wet-weighing.

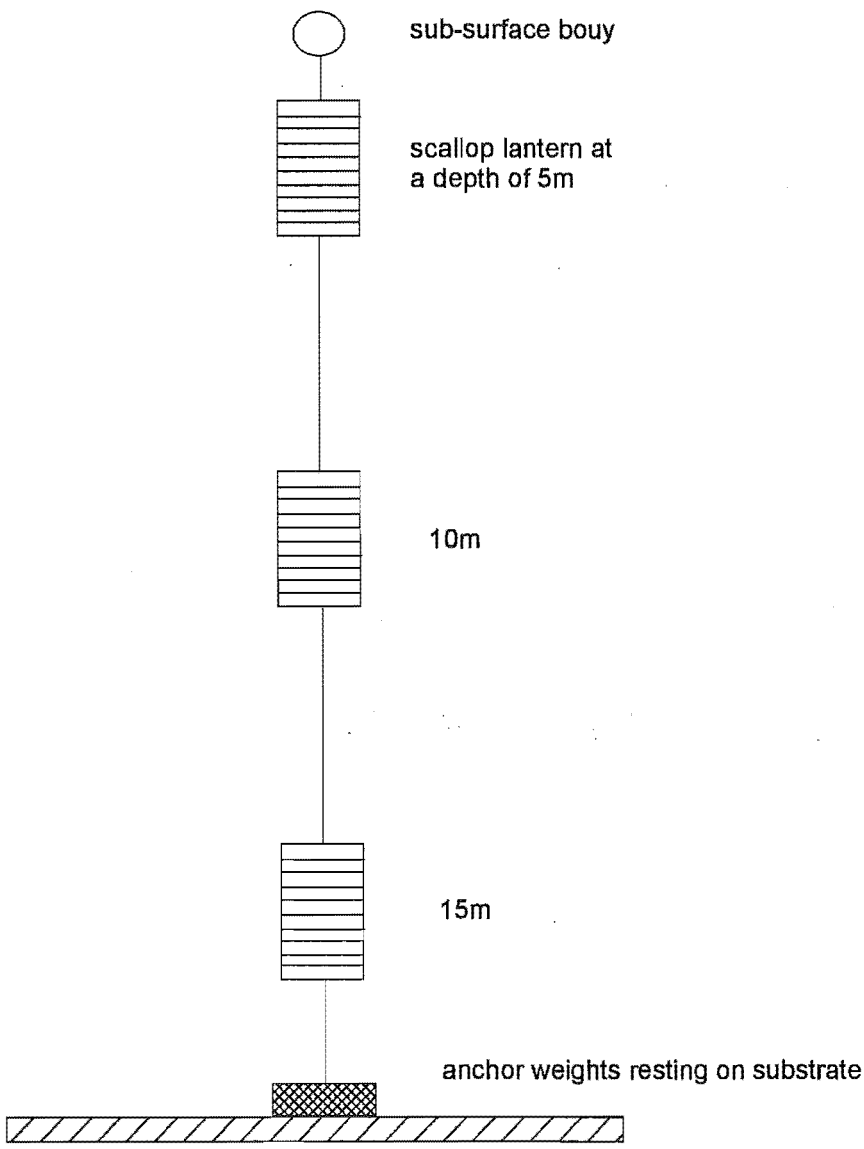


Figure 3.2. Schematic diagram of the culture array used for farming sponges experimentally at the low and high exposure sites; scallop lanterns were placed only at 5m and 10m depths at the moderate exposure site.

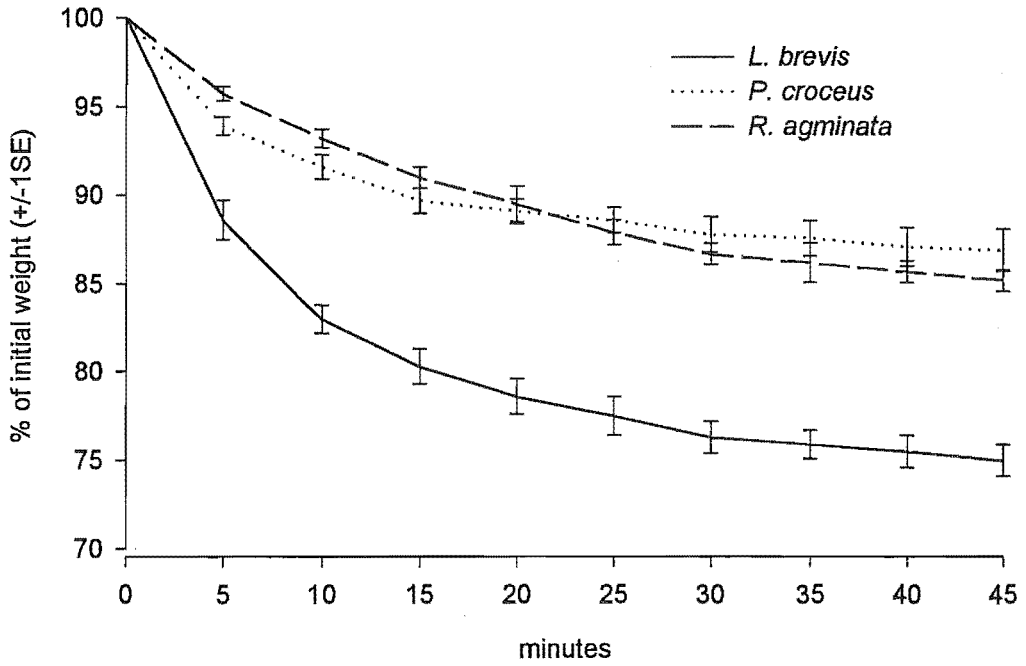


Figure 3.3. Percentage change of initial weight over time for explants of *L. brevis*, *P. croceus* and *R. agminata*. Explants were 16cm^3; n=10 for each species.

At the start of each season fifty explants each of *L. brevis* and *P. croceus* were wet-weighed to determine their initial weight (Table 3.1). One-Way ANOVA's determined that initial explant weights were significantly different between seasons for both *L. brevis* ($F_{df(4,245)}$=7.36, P<0.001) and *P. croceus* ($F_{df(3,196)}$=15.37, P<0.001). These differences in initial weights between seasons resulted from experimental error in cutting the explants to slightly different sizes. An ecological study determined that small differences in sponge size do not influence their growth or survival (Chapter 2). However, these seasonal differences prevented use of final explant weight to compare environmental factors. Instead, growth of individual explants was compared by adjusting for their initial weights:

explant growth = final weight / mean initial weight x 100

Explants were monitored during each farming experiment, their survival recorded and any biofouling noted. Any fouling organisms such as algae and bushy bryozoans that could have reduced the water flow to explants were removed from the lanterns.

Table 3.1. Mean initial explant weight (±1SE) of *L. brevis* and *P. croceus* farmed in each season.

Season	*L. brevis*	*P. croceus*
autumn `97	17.1 (0.6)	N/A
winter `97	14.5 (0.5)	13.7 (0.4)
spring `97	16.9 (0.5)	16.6 (0.5)
summer `98	17.7 (0.5)	17.1 (0.5)
autumn `98	15.4 (0.5)	13.9 (0.5)

3.2.1.4. Monitoring bioactivity

Explant extracts of *L. brevis* and *P. croceus* were analysed against a P388 murine leukaemia screen to determine if season, exposure or depth affected explant bioactivity. Two grams of explant tissue from each treatment was sent to the Chemistry Department at the University of Canterbury for analysis. Each 2g sample consisted of 0.4g sub-samples cut from 5 randomly chosen explants. If an explant looked diseased or unhealthy another was chosen. If there were not 5 explants in a treatment equal portions of the remaining explants to make 2g were taken. Each 0.4g sub-sample included ectosome and choanosome tissue. The chemical analysis procedure is described in Chapter 2.

For both *L. brevis* and *P. croceus*, 2 samples of each major environmental treatment (e.g. winter `97, high exposure, or 10m) were analysed. To compare between seasons, the 2 samples consisted of explants farmed at a depth of 10m from the high and moderate exposure. To compare between exposures and depths, the 2 samples were from explants farmed in winter and spring. The other factor (e.g. exposure when comparing depth) was kept constant. For each sample, the explants from the 3 lanterns in each treatment were pooled. Because only two samples were analysed due to high cost there were insufficient replicates to compare statistically between treatments. Instead, the range of the IC_{50} scores of the 2 samples from each treatment is graphed. Although this range does not show the full variation within a treatment, treatments are considered different if their bioactivity ranges do not overlap.

Because of the costs involved, it was not possible to analyse several replicate explants per treatment individually. However, to gain a greater understanding of the variability in bioactivity for each species the following was done. To examine inter-explant variability, five explants in one scallop lantern were analysed individually. Each explant sample consisted of five 0.4g tissue samples randomly cut from the explant. To examine bioactivity between lanterns within treatments, explants were analysed from each of the three lanterns situated at one depth at one exposure. Finally, to gauge the reliability of the assay method one sample was assayed five times.

3.2.1.5. Statistical analysis

Analysis of variances were used to determine statistically the effect of season, exposure and depth on the growth and survival of *L. brevis* and *P. croceus*. Because the overall design of the experiment was not orthogonal, several ANOVA's were run for the growth and survival of each species. The main analysis compared between: the seasons, winter `97, spring `97, summer `98 and autumn `98; the exposures, low, moderate and high; and the depths, 5 and 10m. The greater depth range (5-15m) at the low and high exposure was examined in a separate analysis. However, only the effect of depth and any significant interaction it has with another environmental factor (e.g. season, exposure) is described. For *L. brevis* only, the farming response was also compared between autumn `97 and autumn `98 using the data from the 3 exposures and the 5 and 10m depths. After each ANOVA, all significant factors were further tested with the *a posteriori* Tukey-Kramer Multiple Comparison test to determine which treatments were significantly different.

3.2.1.6. Physical conditions

As one measure of environmental condition, water temperature was recorded using temperature data loggers, between seasons, exposures and depths. Water movement was also measured between exposures and depths by comparing the erosion of plaster-of-paris discs. These discs erode at a consistent rate relative to water movement, and therefore give a qualitative measure of water movement (Jokiel and Morrissey 1993, Thompson and Glenn 1994). At each exposure one disc was tied to the top of each scallop lantern on 2 arrays. Diver safety and the requirement to transplant all discs within a small window of time so that they experience equal exposure to weather and tides prevented use

of the full 3 arrays at each exposure. The need for suitable diving weather restricted this experiment to a fine spell of settled weather, otherwise discs could not be retrieved. Discs were left in the water for 50 hours and results represent a conservative estimate of relative water movement between exposures and depths.

3.2.2. Reciprocal transplant of *Polymastia croceus*

To test the effect of farming *P. croceus* in locations distant from where it was collected a reciprocal transplant experiment was done. In September `97, 12 sponges were collected from the south coast of Wellington and cut into 64 cube-shaped explants, approximately 27cm^3 in size. 32 explants were left in Wellington, stored in tanks with running seawater, and the rest were flown in water-filled buckets to Leigh, ~700km north (Fig. 1.2). At Leigh, another 12 sponges were collected and cut into 64 explants of 27cm^3. The 32 Wellington explants and 32 Leigh explants were placed into scallop lanterns in the Sponge Garden situated in the Cape Rodney to Okakari Point Marine Reserve (38° 16'S, 174° 48"E). On return to Wellington the remaining 32 Wellington and 32 Leigh explants were transplanted into scallop lanterns situated in Mahanga Bay. At each location 2 arrays, 15m apart, with scallop lanterns situated at depths of 5 and 10m were setup. Into each lantern 8 explants sourced from each location were transplanted into a randomly chosen and separate compartment. This experiment ran for 73 days, from 23 September `97 to 5 December `97.

3.3. Results

3.3.1. Farming *Latrunculia brevis* and *Polymastia croceus* in different seasons, exposures and depths

3.3.1.1. Summer transplant

In late summer `98 the toxic algae *Gymnodinium brevisulcatum* bloomed in Wellington Harbour killing many marine organisms (Chang 1999). Explant monitoring shortly before the bloom recorded high survival of *P. croceus* at all exposures (Table 3.2). However, by the end of summer only *P. croceus* explants at the high exposure site, where *G. brevisulcatum* was present in low numbers (Chang, personal communication), survived. In contrast, most *L. brevis* were dead before the bloom (Table 3.2) and none survived by the end of summer. Although harmful algal blooms are a seasonal occurrence, more common in spring and summer (Bricelj and Lonsdale 1997), the summer season was

excluded from the statistical analysis because large-scale toxic algae blooms rarely occur (Chang, personal communication) and the objective was to examine farming in "normal" conditions. The great mortality during summer did not allow the use of these data in a full ANOVA model. Unless mentioned all analysis is for winter `97, spring `97 and autumn `98.

Table 3.2. Percent survival of *L. brevis* and *P. croceus* at each exposure in summer `98, several weeks before the toxic algal bloom.

Exposure site	*L. brevis*	*P. croceus*
low	21	93
moderate	34	84
high	18	91

3.3.1.2. *Latrunculia brevis*

Explant growth varied between seasons (Table 3.3a). Overall growth was greatest during winter, when most explants surpassed their initial weight (average increase of 1g), and least during autumn (Fig. 3.4). Neither exposure nor depth had an overall significant effect on explant growth (Table 3.3a). However, increasing exposure generally promoted better explant growth (Fig. 3.4). Overall final weight was 87% of initial explant weight at the high exposure site, 83% at the moderate exposure site, and 75% at the low exposure site. There was also a significant exposure*depth interaction (Table 3.3a). Growth was greatest at 10m in the high exposure site location but at 5m in the more sheltered exposures.

L. brevis explants farmed for two months in similar conditions varied greatly in growth. For example, final weights of explants farmed across all exposures in winter `97 ranged from 4-30g (Fig 3.5). Within each exposure, final weights were evenly distributed between the 3 arrays. In addition, growth within a single compartment of a scallop lantern could be highly variable, with some explants doubling their weight while others shrank. Some explants also partially fused with their neighbours to form a single sponge, but individual explants could still be distinguished and were weighed separately.

Table 3.3. Analysis of variance for growth and survival of farmed explants of *L. brevis* between different environmental conditions. GLM ANOVA used to analyse data. Season compares winter, spring and autumn '98 only. Depth compares 5 and 10m only. To meet assumptions, growth data were log transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
a) growth					
season	2	1.326	0.663	26.4	<0.0001*
exposure	2	0.163	8.131	3.24	0.054
depth	1	0.00743	0.00743	0.3	0.591
season*exposure	4	0.0654	0.0163	0.65	0.631
season*depth	2	0.00409	0.00204	0.08	0.922
exposure*depth	2	0.217	0.108	4.31	0.023*
season*exposure*depth	4	0.0492	0.0123	0.49	0.744
lantern (season*exposure*depth)	28	0.703	0.0251	1.54	0.051
error	169	2.758	0.0163		
total	214	5.724			
b) survival					
season	2	158.9	79.4	8.24	0.009*
exposure	2	17.59	8.79	0.91	0.436
depth	1	0.0185	0.0185	0.00	0.966
season*exposure	4	70.63	17.66	1.83	0.207
season*depth	2	1.815	0.907	0.09	0.911
exposure*depth	2	8.037	4.019	0.42	0.671
season*exposure*depth	4	4.629	1.157	0.12	0.972
array(season*exposure*depth)	9	86.83	9.648	4.78	0.0007*
error	27	54.5	2.019		
total	53	403			

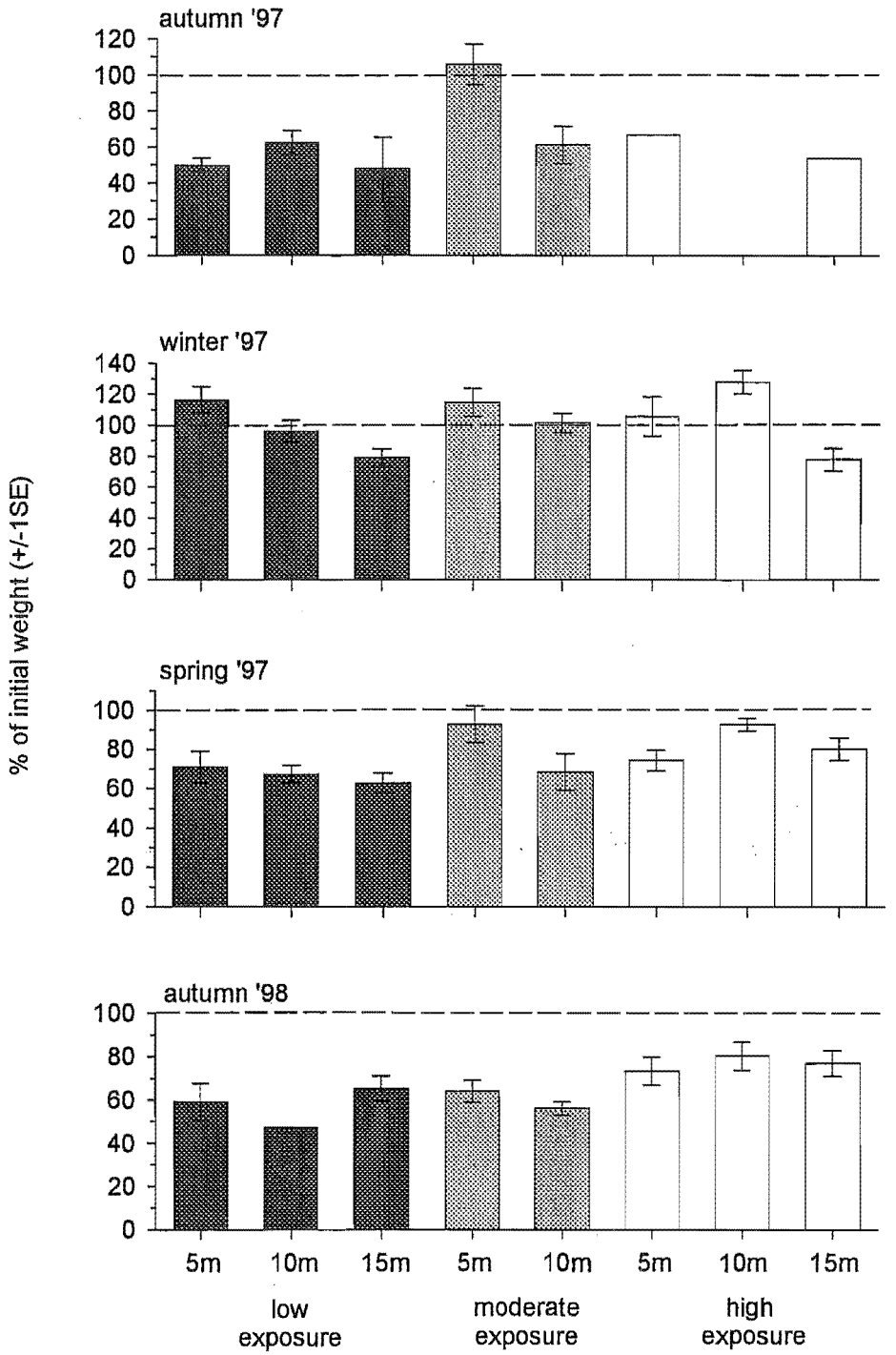


Figure 3.4. Growth (% of initial weight) after 2 months of *L. brevis* farmed in different seasons, exposures and depths. All explants died in summer '98. Dashed lines represent initial weight (100%). Error bars represent variation between explants.

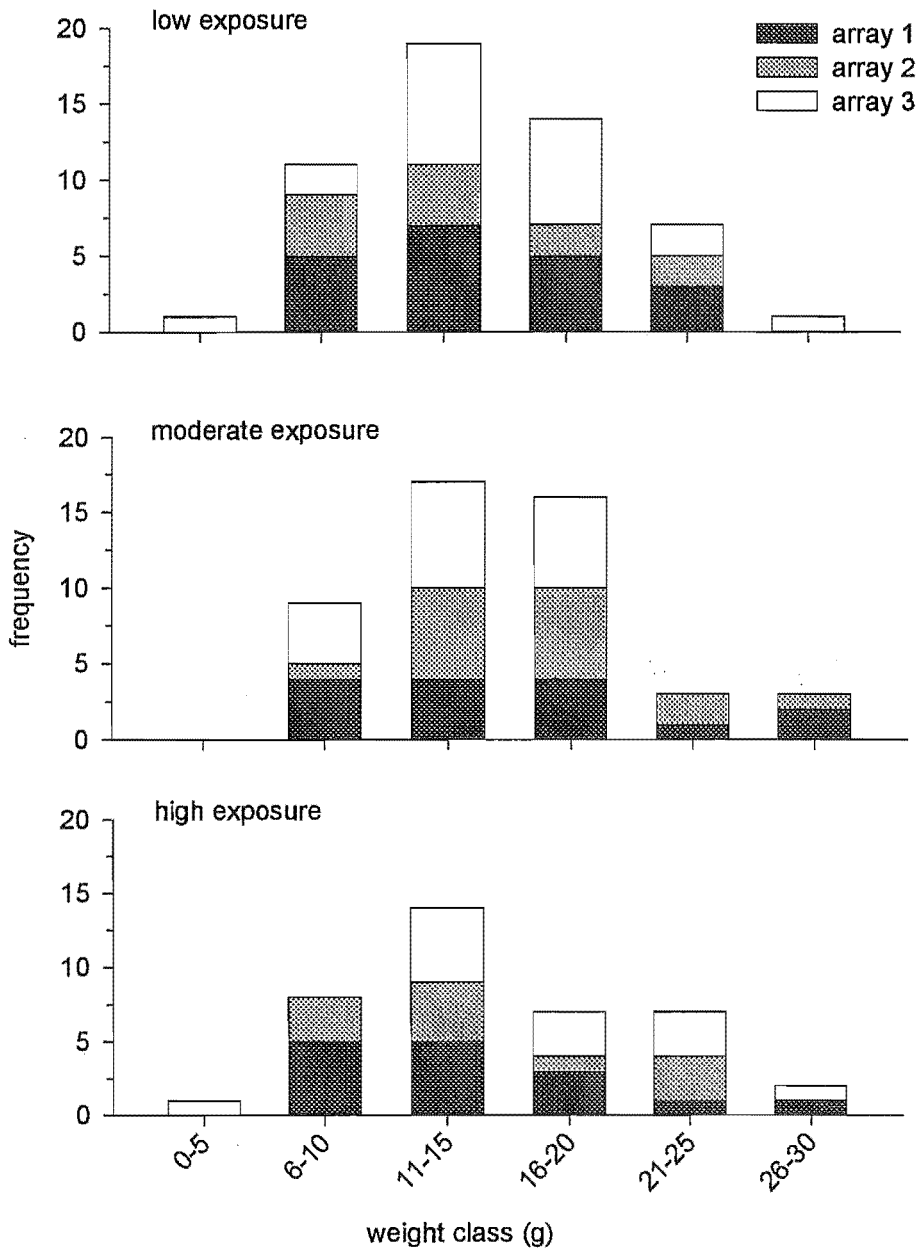


Figure 3.5. Final weight frequency distribution of *L. brevis* farmed at the low, moderate and high exposures in winter '97, and separated into arrays. Mean initial explant weight was 14.5g.

Survival of *L. brevis* was greatest during winter `97 when growth was best (Table 3.3b, Fig. 3.6). Survival was mostly <20% during the autumn months (Fig. 3.6). Survival did not vary between exposure or depth, but did vary significantly between arrays (Table 3.3b). This indicates that there were some very localised influences on survival, and it may relate to the levels of biofouling which can vary over small spatial scales.

Dead explants of both *L. brevis* and *P. croceus* were often covered with an unidentified white fungal film. The sponge tissue beneath this film was black and pungent. No *L. brevis* explants became fouled by macro-organisms at any time.

The statistical analysis that examined the greater depth range determined that neither growth ($F_{df(2,29)}$=1.51, P=0.237) nor survival ($F_{df(2,36)}$=0.08, P=0.922) of *L. brevis* varied between 5, 10 and 15m (Fig. 3.4&6). There were also no interaction effects.

In addition, the farming response of *L. brevis* was compared between autumn `97 and autumn `98. Statistical analysis determined that for survival there was a significant year*exposure interaction ($F_{df(2,24)}$=6.87, P=0.0044) with greater survival at the high exposure site in `98 (21%) than in `97 (2%) (Fig. 3.6). There were no other significant interactions. Unlike explant survival, growth was similar between years ($F_{df(1,45)}$=1.74, P=0.194) and there were no interaction effects (Fig. 3.4).

The low variation in bioactivity between the five assays of one sample (Table 3.4a) is a favourable result as it indicates that the bioassay method generates precise bioactivity scores for *L. brevis*. However, the variation of bioactivity between explants farmed within and between lanterns (Table 3.4b,c) indicates explants can react differently to the same stimuli within a treatment.

Table 3.4. Mean ($\bar{x}$), standard error (SE) and coefficient of variation (CV) of bioactivity (ng/ml) of *L. brevis* between a) 5 assays of one sample, b) 5 explants farmed in one scallop lantern, and c) between the 3 lanterns situated at one depth in one exposure.

	$\bar{x}$	SE	CV
a) 5 assays	1583.5	243.9	15.4
b) within lantern	327.4	168.6	51.5
c) between lanterns	111.6	23.5	21

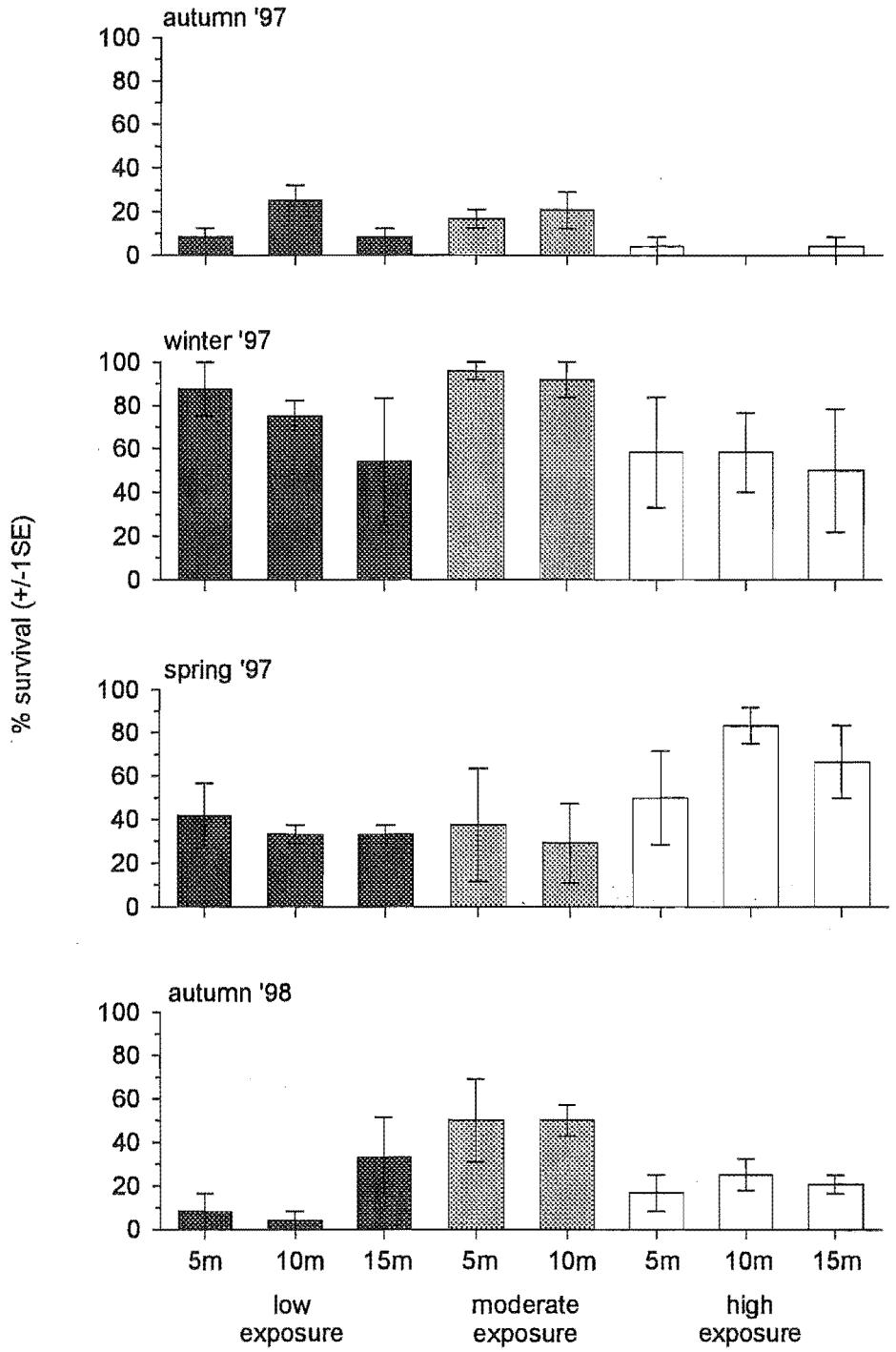


Figure 3.6. Percentage survival after 2 months of *L. brevis* farmed in different seasons, exposures and depths. All explants died in summer '98. Errors bars represent variation between lanterns.

Explant bioactivity was very high (low IC_{50}) in all seasons, exposures and depths (Fig. 3.7). Between seasons, there is considerable overlap of IC_{50} scores (Fig. 3.7), indicating that bioactivity of *L. brevis* is similar regardless of the season of farming. There was also no difference in bioactivity between autumn `97 and autumn `98. Bioactivity was also similar between explants farmed at each exposure and at each depth (Fig. 3.7).

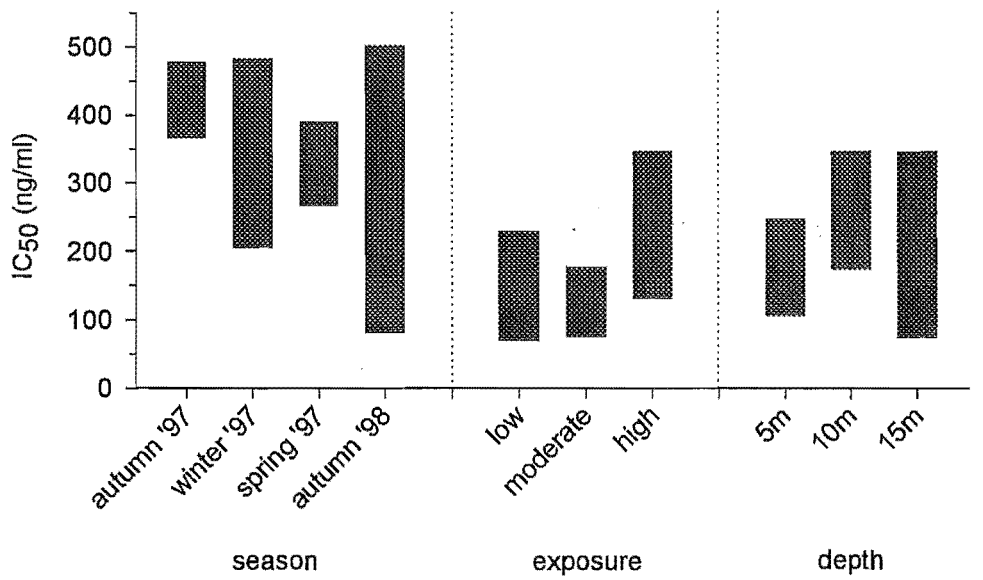


Figure 3.7. Bioactivity (IC_{50} range) of *L. brevis* farmed in different seasons, exposures and depths (n=2 for each treatment). As IC_{50} decreases activity increases. IC_{50} <1500 is considered very active.

3.3.1.3. *Polymastia croceus*

Statistical analysis of growth showed a significant season*exposure*depth interaction (Table 3.5a), implying that growth of explants depends on the time and the place they are farmed. Greatest growth occurred for explants farmed during spring at a depth of 5m at the low exposure site and at 10m at the high exposure site (Fig. 3.8). These explants grew on average by 8g or 50% of their initial weight in two months. Comparing between seasons, overall growth was greatest during spring (Fig. 3.8). Explants farmed at high exposure grew the most during each season (Fig. 3.8). Overall final weight was 125% of initial explant weight in the high exposure site, 102% in the moderate exposure site, and 104% in the low exposure site.

As for *L. brevis*, explants of *P. croceus* farmed for two months in similar conditions varied greatly in growth. For example, final weight of explants farmed across all exposures in spring `97 ranged from 8-46g (Fig. 3.9). Within each exposure, final weights were evenly distributed between the 3 arrays. Growth within a single compartment of a scallop lantern could also be highly variable, with some explants doubling their weight while others shrank. Some explants of *P. croceus* also partially fused with their neighbours to form a single sponge. Like *L. brevis*, these explants were weighed separately. In addition, no explant was seen to be fouled by macro-organisms.

Survival of *P. croceus* did not vary between seasons, exposures or depths (Table 3.5b, Fig. 3.10) but did vary significantly between arrays, possibly indicating some localised influences on survival. High percentage survival of *P. croceus* in an array did not correlate with high survival of *L. brevis* (Canonical Correlation: r=0.012, P=0.92).

The statistical analysis that examined the greater depth range determined that for growth there was a significant season*exposure*depth interaction ($F_{df(4,35)}$=6.14, P=0.0007). Growth was greatest during spring at a depth of 5m at the low exposure site and at 10m at the high exposure site (Fig. 3.8). For survival, there was a significant season*depth interaction ($F_{df(4,36)}$=4.21, P=0.002), with relatively poor survival at 15m in winter (Fig. 3.10).

Table 3.5. Analysis of variance for growth and survival of farmed explants of *P. croceus* between different environmental conditions. GLM ANOVA used to analyse data. Season compares winter, spring and autumn only. Depth compares 5 and 10m only. To meet assumptions, growth data were log transformed. Prob: * = significant. Note: Degrees of freedom of some factors differ from *L. brevis* because of the greater survival of *P. croceus* explants.

Factor	DF	SS	MS	F-ratio	Prob
a) growth					
season	2	0.290	0.145	14.09	<0.0001*
exposure	2	0.608	0.304	29.56	<0.0001*
depth	1	0.00888	0.00888	0.86	0.359
season*exposure	4	0.203	0.0507	4.94	0.0028*
season*depth	2	0.176	0.0882	8.58	0.0009*
exposure*depth	2	0.187	0.0933	9.08	0.0006*
season*exposure*depth	4	0.370	0.0926	9.01	<0.0001*
lantern (season*exposure*depth)	36	0.370	0.0103	0.84	0.727
error	351	4.279	0.0122		
total	404	6.551			
b) survival					
season	2	6.037	3.019	1.6	0.255
exposure	2	4.592	2.296	1.22	0.341
depth	1	0.296	0.296	0.16	0.701
season*exposure	4	5.185	1.296	0.69	0.619
season*depth	2	0.037	0.0185	0.01	0.990
exposure*depth	2	1.037	0.519	0.27	0.766
season*exposure*depth	4	2.296	0.574	0.30	0.868
array(season*exposure*depth)	9	17	1.889	3.92	0.0027*
error	27	13	0.481		
total	53	49.481			

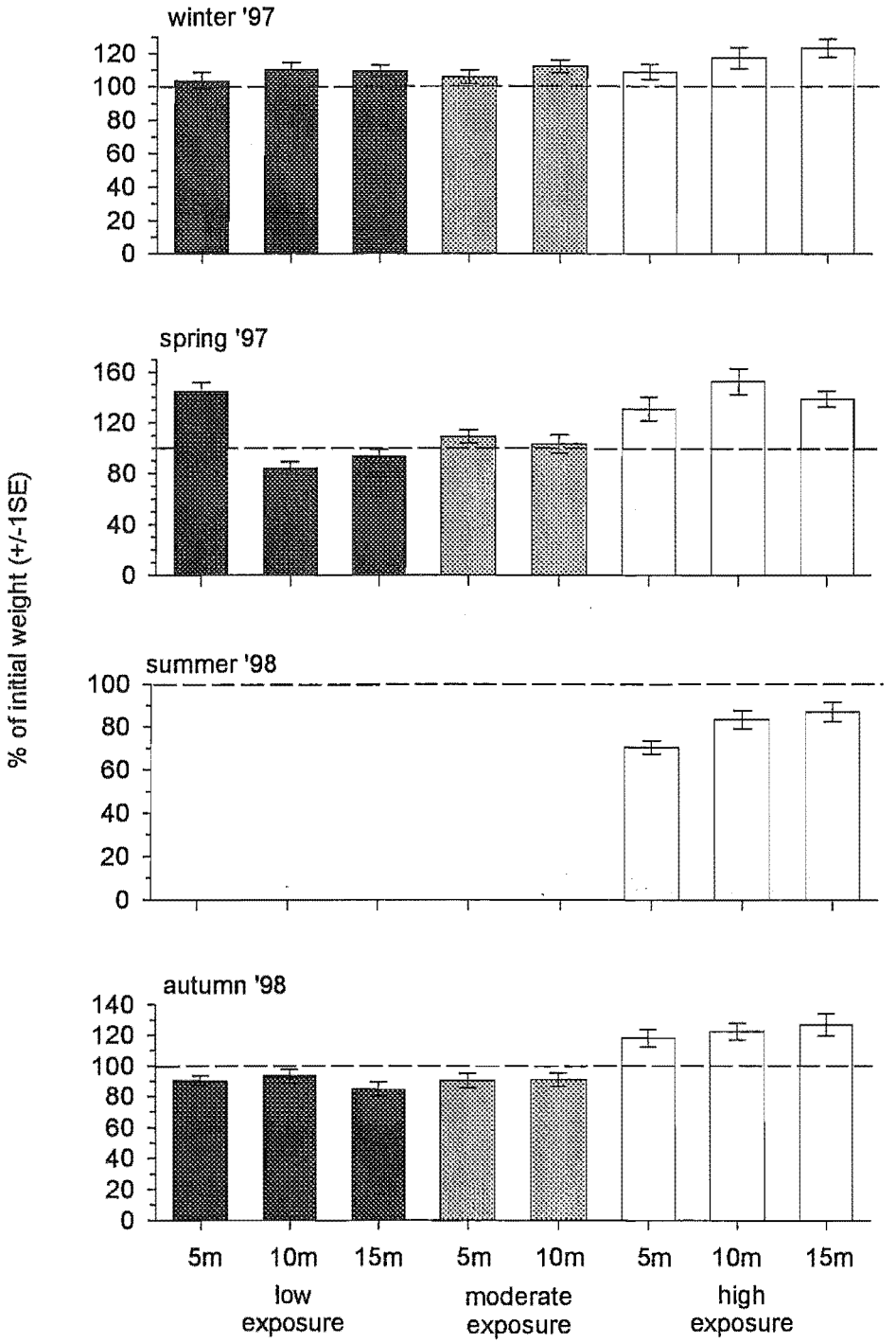


Figure 3.8. Growth (% of initial weight) after 2 months of *P. croceus* farmed in different seasons, exposures and depths. All explants died at the low and moderate exposures in summer '98. Dashed lines represent initial weight (100%). Error bars represent variation between explants.

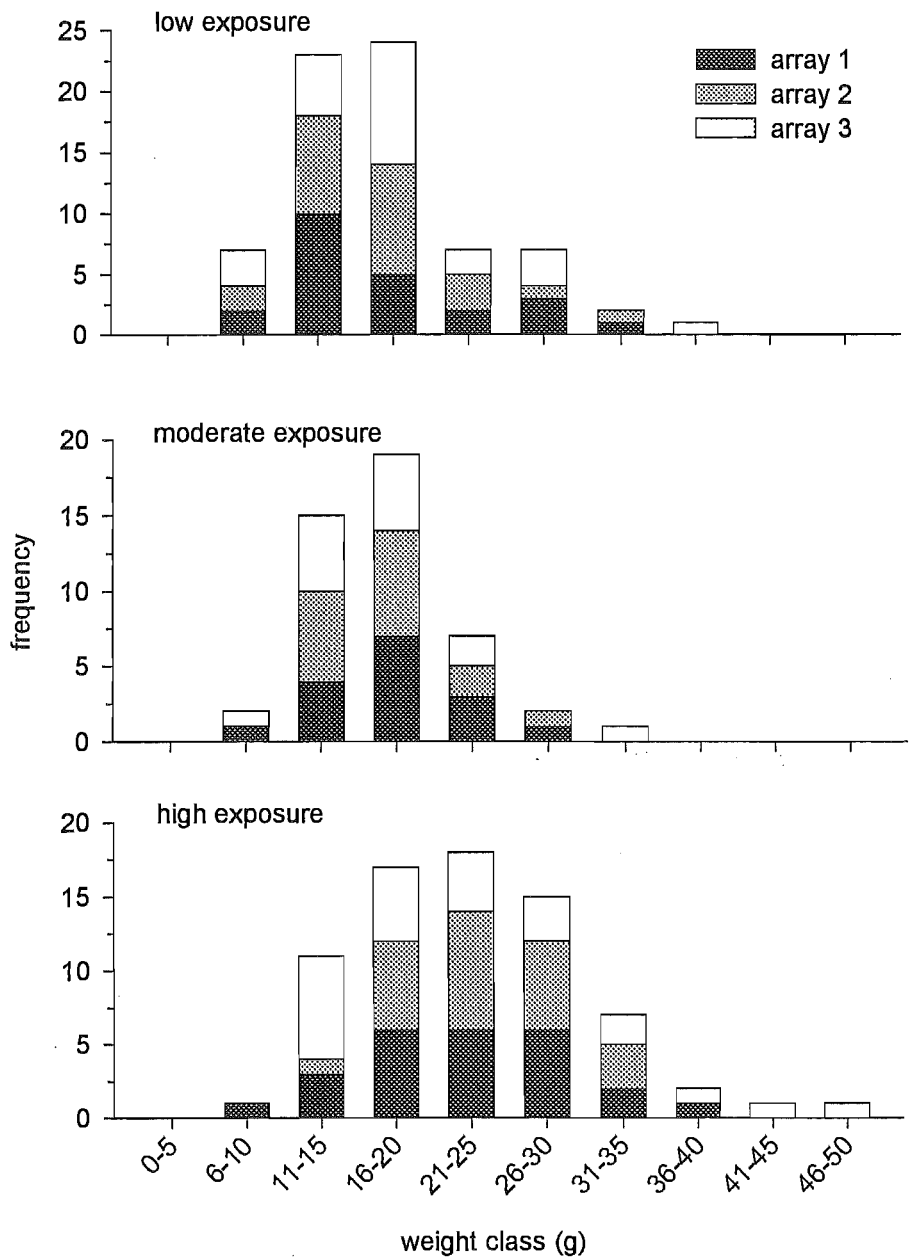


Figure 3.9. Final weight frequency distribution of *P. croceus* farmed at the low, moderate and high exposures in spring '97, and separated into arrays. Mean initial explant weight was 16.6g.

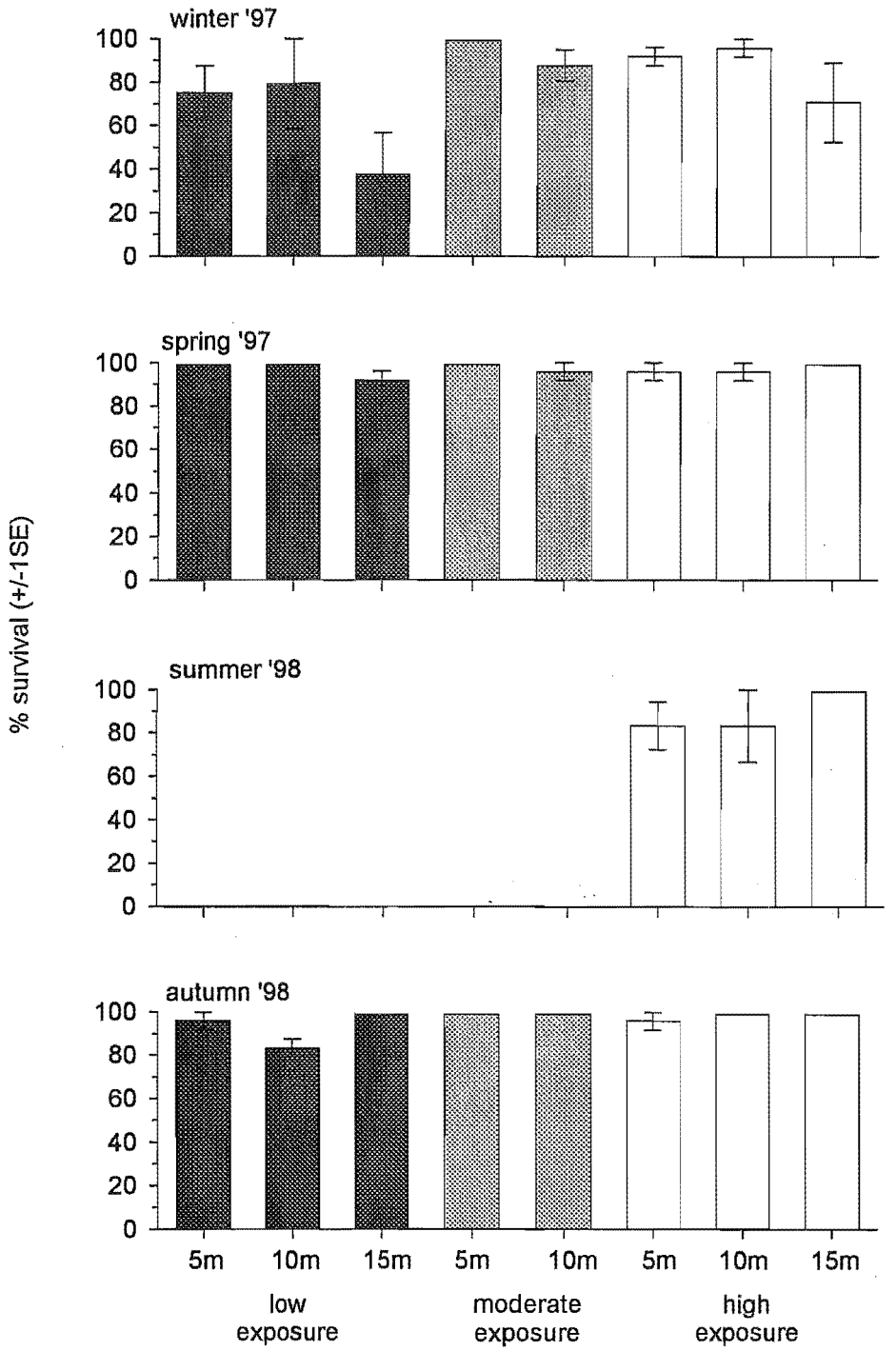


Figure 3.10. Percentage survival after 2 months of *P. croceus* farmed in different seasons, exposures and depths. Errors bars represent variation between lanterns.

The low variation in bioactivity between the five assays of one sample (Table 3.6a) indicates that the bioassay method generates precise bioactivity scores for *P. croceus*. However, great variation of bioactivity between explants of *P. croceus* farmed within and between lanterns (Table 3.6b,c) indicates explants can react differently to the same stimuli within a treatment.

Table 3.6. Mean ($\bar{x}$), standard error (SE) and coefficient of variation (CV) of bioactivity (ng/ml) of *P. croceus* between a) 5 assays of one sample, b) 5 explants farmed in one scallop lantern, and c) between the 3 lanterns situated at one depth in one exposure.

	$\bar{x}$	SE	CV
a) 5 assays	177	9.4	5.3
b) within lantern	602	431.3	71.6
c) between lanterns	306.7	169.3	55.2

Explant bioactivity was high and similar between seasons, exposures and depths (Fig. 3.11). Interestingly, the most active sample (IC_{50}=47) was taken from explants farmed at the high exposure site during summer when the toxic algal bloom was devastating marine life in Wellington Harbour (Chang 1999). Such a low IC_{50} score is considered "extremely potent" (Lill et al. 1995).

3.3.1.4. Comparing the farming response between *Latrunculia brevis* and *Polymastia croceus*

Comparing the two sponge species, *P. croceus* had the most promising growth and survival. Overall, over 90% of *P. croceus* explants survived with an average growth of 112% or weight increase of 2g per explant each two month period. Growth was greatly affected by the interaction of season*exposure*depth. Except for poor survival at 15m in winter, survival was similar between seasons, exposures and depths. In contrast, *L. brevis* had a poor farming response. Overall, only 50% of *L. brevis* explants survived with an average weight loss of 3g per explant. The season of farming had the greatest influence on the growth and survival of *L. brevis*.

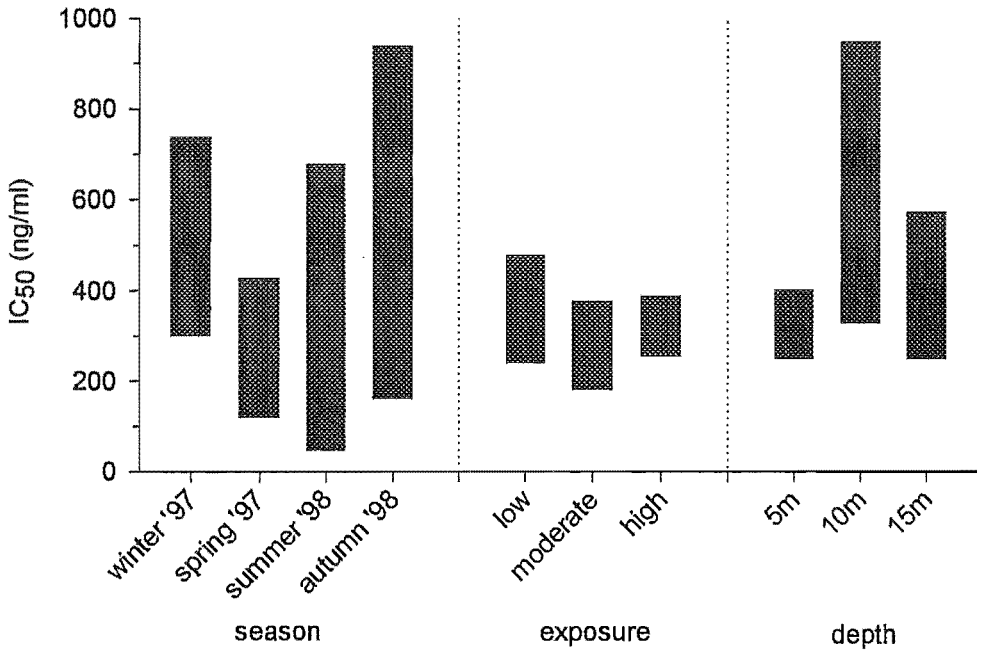


Figure 3.11. Final bioactivity (IC_{50} range) of *P. croceus* farmed in different seasons, exposures and depths (n=2 for each treatment). As IC_{50} decreases activity increases. IC_{50} <1500 is considered very active.

3.3.1.5. Physical conditions

The overall average water temperature was lowest during winter (August, 9°C) and highest during summer (February, 20°C) (Fig. 3.12a). There was some variation between exposures especially during late autumn when there was a 1.5°C difference (Fig. 3.12b). There were only minor differences between depths (Fig. 3.12c). These temperature results agree with other studies which suggest water is well mixed in Wellington Harbour (Maxwell 1955, Booth 1975, Heath 1977).

Erosion of the plaster-of-paris discs differed significantly between exposures ($F_{df(2,13)}$=5.43, P=0.019) and depths ($F_{df(2,13)}$=6.2, P=0.013). For both factors, separate One-Way ANOVA tests were run because of the non-orthogonal design of the experiment. Erosion tended to decrease from the high to the low exposure site and also decreased with depth within each exposure (Fig. 3.13). This indicates decreasing water movement from the high to the low exposure site, and decreasing water movement with depth within each exposure.

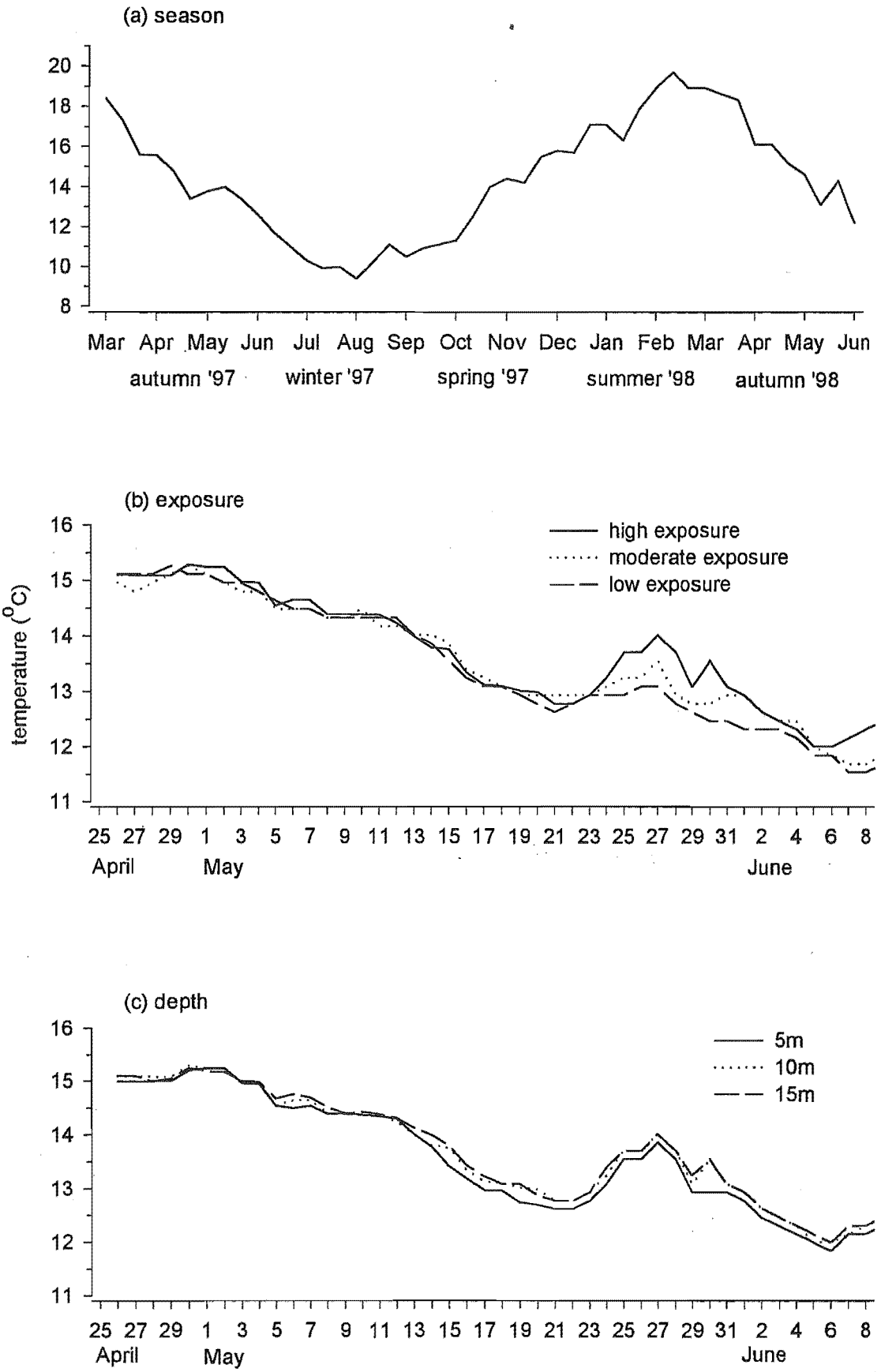


Figure 3.12. Water temperature in Wellington Harbour between seasons, exposures and depths. Exposure and depth compared during autumn '98. Depth compared at the high exposure site during autumn '98.

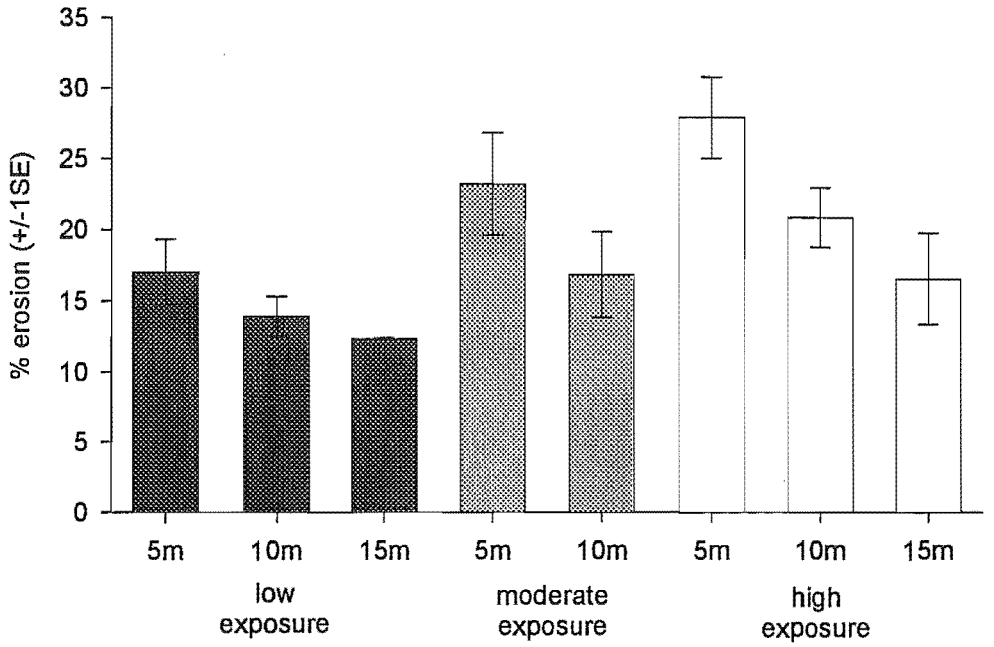


Figure 3.13. Water movement, measured by percent erosion of plaster-of-paris discs, among exposures and depths. Great erosion signifies greater water movement.

3.3.2. Reciprocal transplant of *Polymastia croceus*

The major result was that Leigh sponges grew better at Leigh and the Wellington sponges grew better in Wellington (Fig. 3.14). This is supported by the significant interaction term of farming location*explant source (Table 3.7a). It is unlikely these results reflect differences in handling stress between explants collected locally or from the other region because transport times between locations were less than 4 hours and all explants transported were kept in chilled aerated water to further minimise their stress. Growth at each farming location was similar between depths (Table 3.7a). Survival was high (78%) overall and did not vary between farming locations, depths, explant source or any interaction (Table 3.7b).

Tissue structure of sponges sourced from the two locations was noticeably different, with Leigh sponges being softer and fleshier. Thin tissue sections of sponges from each location were examined using the taxonomic review of the genus *Polymastia* by Kelly-Borges and Bergquist (1997). This determined that all sponges were of the species

P. croceus. Tissue structure had not noticeably changed by the end of the experiment, regardless of where sponges were grown.

Table 3.7. Analysis of variance for growth and survival of *P. croceus* in the reciprocal transplant experiment. GLM ANOVA used to analyse data. To meet assumptions, growth data log transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
a) growth					
farming location	1	0.0012	0.0012	0.1	0.758
explant source	1	0.0132	0.0132	1.14	0.317
depth	1	0.0313	0.0313	2.71	0.138
location*source	1	0.1135	0.1135	9.83	0.014*
location*depth	1	0.0017	0.0017	0.1	0.759
source*depth	1	0.00089	0.00089	0.08	0.787
location*source*depth	1	0.000008	0.000008	0.00	0.979
array(location*source*depth)	8	0.0925	0.0156	0.93	0.493
error	84	1.0396	0.0124		
total	99	1.2935			
b) survival					
farming location	1	0.0244	0.0244	0.49	0.504
explant source	1	0.0009	0.0009	0.02	0.892
depth	1	0.0088	0.0088	0.18	0.685
location*source	1	0.0791	0.0791	01.59	0.243
location*depth	1	0.0088	0.0088	0.18	0.685
source*depth	1	0.0488	0.0488	0.96	0.356
location*source*depth	1	0.0009	0.0009	0.02	0.892
error	8	0.3984	0.0498		
total	15	0.5693			

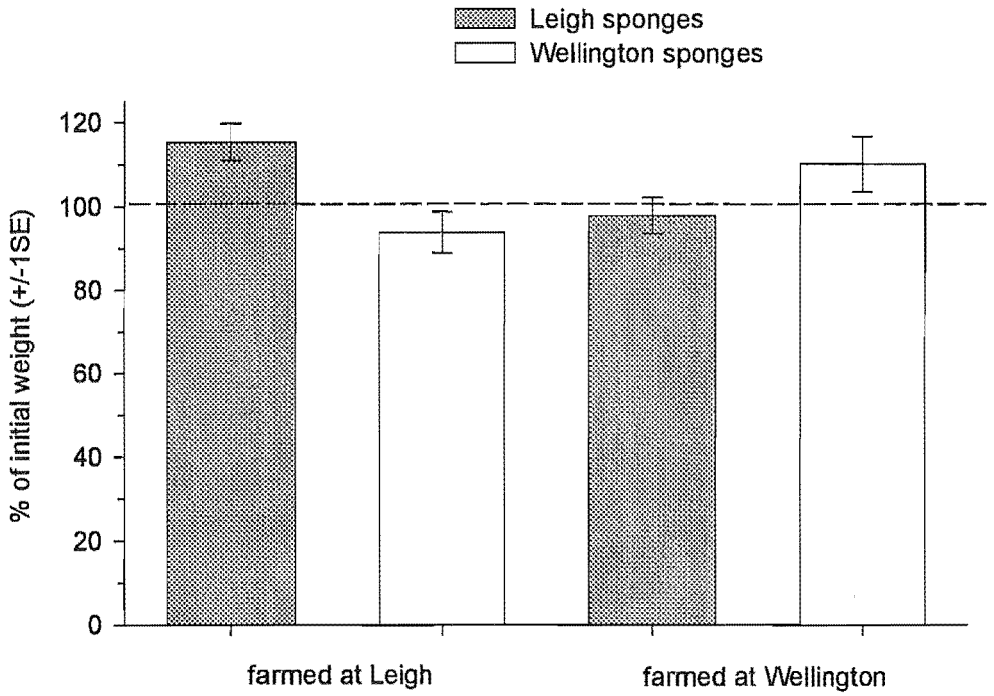


Figure 3.14. Growth (% of initial weight) of *P. croceus* in a reciprocal transplant between Leigh and Wellington. Dashed line represents initial weight (100%).

3.4. Discussion

The great variation in farming response shown between *Latrunculia brevis* and *Polymastia croceus* is something which is common to all studies which have farmed more than one sponge species (Verdenal and Vacelet 1990, Duckworth et al. 1997, Pronzato et al. 1999). This variation between species probably stems from physiological differences in their ability to survive transplanting and reorganise their cut tissues into fully functional explants (Wilkinson and Vacelet 1979, Verdenal and Vacelet 1990). Why *P. croceus* is physiologically better equipped to survive damage than *L. brevis* is unknown.

For *L. brevis*, this study indicates that its ability to survive transplanting and to reorganise its cut tissue is greatly affected by seasonal differences in water temperature. An ecological study determined that survival of wild *L. brevis* is similar over all seasons (Fig. 2.1), but survival of farmed *L. brevis* was greatest in winter when the water

temperature was lowest (9°C). Cooler water promotes survival in several ways. Respiration is lower in cooler water (Barthel and Theede 1986, Burlando et al. 1992, Cheshire et al. 1995) and this reduces stress during transplanting. Cooler water also promotes quicker pinacoderm healing (Duckworth et al. 1997) and reduces microbial growth (Hummel et al. 1988, Vacelet et al. 1994), both of which may reduce the chance of infection.

Like survival, seasonal growth differed between farmed and wild *L. brevis*. In '97, farmed *L. brevis* grew only in winter but wild sponges grew in both winter and spring (Fig. 2.3). This difference in growth between farmed and wild sponges indicates that the seasonal conditions affecting survival of *L. brevis* explants also influences their growth. A relationship between survival and growth is not always expected when farming sponges. For example, *Raspailia agminata*, one of three sponges farmed experimentally by Duckworth et al. (1997), had similar survival at depths of 5, 10 and 17m but grew well at 10m only. For *L. brevis*, healthy explants with little stress resulting from cooler water probably divert more energy into growth, which may explain the high growth in winter.

High survival of farmed *P. croceus* in all seasons, including summer before the toxic algae bloom, indicates that ambient water temperature does not influence its ability to heal wounds and survive. Unaffected by the seasonal influence on survival, growth of *P. croceus* explants was probably determined by water temperature and its effect on food abundance. The main food of *P. croceus* is ultraplankton (<10μm) (Bell et al. 1999) which generally increases in abundance as water temperature rises (Fogg 1986, Joint 1986, Waterbury et al. 1986, Tamigneaux et al. 1995). This suggests that the good growth of *P. croceus* farmed in spring, averaging 120% of initial weight, results from the greater availability of food promoted by rising water temperature.

The exposure of sites greatly affected the growth of *P. croceus*. High water movement generally promotes high growth of sponges (Watson 1976, Wilkinson and Vacelet 1979, Verdenal and Vacelet 1990) through increased availability of food. *P. croceus* occurs naturally at the exposed site. The results here indicate that even if similar substrata are provided across many exposures, growth is generally poorest in sheltered conditions where food availability is lowest. In this study, there was a six fold difference in growth of *P. croceus* between the sheltered and exposed sites. For *L. brevis*, growth did not differ significantly between sites although there was a trend toward greater growth

with increasing exposure. Perhaps the two month long farming period allowed insufficient time for noticeable differences in growth to appear for *L. brevis*.

Depth as a main factor did not influence the growth or survival of farmed *L. brevis* or *P. croceus* directly. Although the depth range was only 10m (5-15m) in this study, both species were farmed at a depth shallower than they naturally occur in Wellington. Dive surveys determined that neither species occurs at depths less than 7m. Farming sponges in shallow depths outside their natural range can reduce explant growth and survival probably through exposure to high light levels (Wilkinson and Vacelet 1979, Duckworth et al. 1997). However, similar overall growth and survival of *L. brevis* and *P. croceus* farmed at 5m compared with deeper depths suggests that light intensity is not the major factor determining their upper limit of distribution.

This study suggests that both species may be excluded from depths less than 7m around Wellington by the effect of very high water movement in shallow depths. Both *L. brevis* and *P. croceus* farmed at the high exposure site, adjacent to where they naturally occur, grew poorest at the shallow depth of 5m where water movement is greatest. Explant growth at the moderate and low exposures, however, was greatest at 5m. Studies that have explored the distribution and abundance of subtidal sponge species over depth have generally found that encrusting sponges are more common at shallow depths while massive, discrete sponges are more common at deeper depths (Schmahl 1990, Robert and Davies 1996). Water movement has been proposed to be a major environmental factor structuring sponge distribution (Reiswig 1973, Wilkinson and Evans 1989, Schmahl 1990, Leichter and Witman 1997). This study provides experimental evidence that very high water movement can affect the growth of massive, discrete sponges, such as *L. brevis* and *P. croceus* negatively. This may be one factor that prevents their distribution into shallow exposed areas.

Transplanting and farming sponges in locations more suitable for commercial production is required for successful sponge aquaculture. The reciprocal transplant experiment showed that *P. croceus* sponges can be collected from a location ~700km away from the farm site and still survive. Poorer growth of relocated explants probably resulted from inappropriate tissue structure for the prevailing environmental conditions. For example, Palumbi (1984, 1986) discovered that the tissue structure of *Halichondria panicea* differed between habitat exposures, with individuals living in high-wave habitats having stronger, stiffer tissue than individuals in low-wave habitats. He also found that

individuals transplanted between habitats grew poorly until their tissue adapted to the prevailing conditions. In this study, *P. croceus* from Wellington had noticeably stiffer tissue than those from Leigh, which may have contributed to the poorer growth of relocated explants in an unfamiliar environment. The transplant experiment ran only for 73 days, with more time, the explants may have adjusted to their new environment and then undergone accelerated growth. Although sponges may need time to adjust to a new environment, these results indicate that it is possible to select, transplant and farm a preferred genotype in a more suitable farming area.

Explants of *L. brevis* and *P. croceus* farmed in similar environmental conditions varied greatly in growth. Variable growth between explants has been found in many sponge species (Moore 1908a, Crawshay 1939, Thompson et al. 1987, Verdenal and Vacelet 1990, Duckworth et al. 1997) and it may result from several factors. Thompson et al. (1987) discovered that genetically identical explants of *Rhopaloeides odaribile* varied greatly in growth and suggested that it may result from differences in initial explant health or nutritional stores. In the present study, 8 explants of each species were placed together in one compartment of each lantern. Johnson (1979) suggested that intraspecific competition for food between individuals of *Clathrina blanca* resulted in some growing while neighbours shrank. Therefore, intraspecific competition between neighbouring explants may also have promoted variation in growth. In addition, minor differences in the handling procedure when cutting and transplanting sponges could induce large differences in growth.

Explant survival also differed greatly between the three arrays situated at each exposure and it may relate to the levels of biofouling which can vary over small spatial scales. Low water flow can reduce survival by either promoting bacterial growth (Hummel et al. 1988), or starving explants of sufficient food or oxygen. Oxygen consumption for sponges is greatest in summer (Burlando et al. 1992) indicating that the effect of biofouling on farming production would be most severe in summer.

Explant survival of *L. brevis* was greater in `98 than in `97, particularly at the exposed site. In addition, growth of wild *L. brevis* and *P. croceus* can vary greatly between years (Fig. 2.3&7). These results indicate that if either species was commercially cultured then tissue production (growth x survival) at a farm may vary from one year to the next. Variation in annual production is common in aquaculture (FAO 1999, aquaculture production statistics from `88-`97). The toxic algal bloom during summer `98

also highlighted the danger to aquaculture of adverse stochastic events that cannot be adequately planned for or controlled.

In aquaculture of sponges to produce bioactive metabolites, another significant source of variation is the influence of the farming environment on metabolite biosynthesis. This was examined in this study by the chemical analysis of explants against a P388 bioactivity screen. These analyses showed that bioactivity of both farmed *L. brevis* or *P. croceus* was similar between seasons. Although this result agrees with wild *P. croceus*, which showed no seasonal variation in bioactivity, farmed sponges were generally more active than wild sponges: from winter `97 to autumn `98 the IC_{50} scores ranged from 190-4675ng/ml for wild sponges but 47-940ng/ml for farmed sponges. Unlike *P. croceus*, wild *L. brevis* show a strong seasonal pattern of bioactivity, being most bioactive in spring (Fig. 2.4). These differences between wild and farmed *L. brevis* and *P. croceus* may result from increased biosynthesis in response to the injury and tissue damage suffered when sponges were cut to make explants. A similar response to injury was also found for the sponge *Aplysina fistularis* which exuded significantly more bioactive metabolites when experimentally damaged (Walker et al. 1985). Elevated biosynthesis in response to damage has also been discovered in some seaweeds (Van Alstyne 1988). In my study, one possibility was that high bioactivity may have resulted from increased biosynthesis due to competitive interactions between explants. However, a separate transplant study determined that when explants of *L. brevis* or *P. croceus* are farmed together and therefore in direct physical contact with each other they have a similar bioactivity to explants farmed individually (Fig. 4.5&6). This indicates that intraspecific competition between explants could not promote the high bioactivity recorded for *L. brevis* and *P. croceus* in this study. Increased bioactivity following damage suggests that one ecological role of the metabolites of *L. brevis* and *P. croceus* is to prevent predation.

Metabolite biosynthesis within a sponge species may also be enhanced in individuals found or grown experimentally at shallow depths, probably to inhibit surface overgrowth of algae (Thompson 1985, Thompson et al. 1987, Kreuter et al. 1991). Although explant bioactivity for *L. brevis* and *P. croceus* was similar between farming depths in this study, possibly because of increased biosynthesis in response to damage obviating any differences, observational evidence suggests that another ecological role for the metabolites is to deter surface fouling. The scallop lanterns used to farm *L. brevis* and *P. croceus* experimentally were quickly and extensively fouled with organisms, in

particular red algae, hydroids (probably *Aglaophenia* sp.)and bushy bryozoans (*Bugula* spp.) but no fouling was seen on any explant.

Large variation in bioactivity between explants of each species, indicating varying concentrations of bioactive metabolites, is a concern for commercial sponge aquaculture for metabolite production. What causes this variation is unclear (Hay 1996, Swearingen and Pawlik 1998) but sponge size may be a contributing factor. Becerro et al. (1997b) discovered that medium sized individuals of *Crambe crambe* are more toxic than small and large individuals.

Several implications for farming are evident from this study which is the first to examine the environmental effect on sponge aquaculture quantitatively. The season of farming can greatly influence the growth and survival of some species such as *L. brevis* probably through seasonal changes in water temperature affecting explant stress and healing after transplanting. This also shows the importance for some species of starting farming experiments in the right season as initial stress may influence final results. For more hardy species such as *P. croceus* the season of farming greatly affects their growth probably through the combination of food availability and water temperature. Exposure or the degree of water movement is also important. Generally farmed sponges grow better in exposed areas where there is a high degree of water movement. However a threshold may exist and if water movement is too great it may damage farmed sponges reducing their growth. In addition, growth and survival may vary greatly between explants farmed in similar environmental conditions possibly because of differences in handling procedure or initial health at the start of farming, or it may result from intraspecific competition. A promising finding of this study was the high survival of sponges grown in areas outside their natural range as this indicates that it is possible to farm in localities which are more suitable for commercial production. This study also suggests that the damage incurred when sponges are cut to make explants may promote their bioactivity for at least two months, and this indicates that the act of transplanting and farming sponges may promote the yield of the target metabolite. Overall, this study provides a good understanding of how the environment may influence the commercial farming of sponges for the production of bioactive metabolites.

Chapter 4. Developing farming structures for commercial sponge aquaculture for metabolite production

4.1 Introduction

A major obstacle to sponge aquaculture for metabolite production is the lack of a suitable farming structure (Shimizu 1995, Osinga et al. 1998), particularly for species with soft morphologies. It is important to develop a farming structure before sponge metabolites are needed in commercial quantities for drug production to guarantee supply and to allow immediate commencement of farming.

There is a long history of research into developing farming structures for sponge aquaculture. In the 1860s, Schmidt tried farming bath sponges in the Adriatic Sea by attaching them to wooden crates (Crawshay 1939). This method failed but several years later his colleague Buccich successfully farmed sponges by hanging them in mid water secured with threaded bamboo poles (Crawshay 1939). Forty years later in Florida, Moore (1908a) grew bath sponges in a similar way using threaded insulated wire instead of bamboo poles to hold explants in mid water. Moore, and later Crawshay (1939), successfully attached and grew sponges on concrete discs. These techniques showed the importance of using appropriate material to promote explant attachment and growth.

After World War II the allied forces investigated Japanese sponge farming practices in the South Pacific (Cahn 1948). They found that the Japanese employed the same methods used by Moore and Crawshay: attaching sponges to concrete discs and threading wire through sponges so that they hung in mid water. Verdenal and Vacelet (1990) and more recently Pronzato et al. (1999) used modern materials (plastics) to adapt this "hanging" method to grow Mediterranean bath sponges.

Until recently all experiments developing farming structures for sponge aquaculture were concerned with bath sponge production. The size and shape of bath sponges determined their market value (Storr 1964, Bergquist and Tizard 1969) which limited the variety of suitable farming structures. In contrast, explant shape has no significance for efficient metabolite production, and consequently there is considerable flexibility in developing new farming methods for metabolite aquaculture. This has been explored, to a limited degree, by Battershill and Page (1996) and Duckworth et al. (1997) who grew sponges with biomedical potential in mesh structures. Their methods differ in the degree of

explant separation. Battershill and Page (1996) grew explants of *Lissodendoryx* n. sp. grouped together in scallop lanterns, while Duckworth et al. (1997) grew explants of *Raspailia agminata* separately in mesh bags.

Sponges have therefore been farmed experimentally on different substrate, inside mesh bags and with wire threaded through them. To develop a farming structure specifically designed for metabolite production it is necessary to examine the farming suitability of each general method, comparing explant growth, survival and bioactivity levels. Within each general method it is necessary to explore the responses of sponge species to the material being used. Important question include: When farming sponges in mesh should they be grown separately or together? What mesh size is best? When farming sponges on a substrate, should natural or artificial materials be used?

Because of the diverse range of sponge morphologies it is impossible to develop one farming structure suitable for all species. The research detailed here focuses on developing a structure (Table 4.1) for farming massive discrete sponges, a morphology which includes many sponge species with metabolites which are in drug trials. Experiments 1-4 are preliminary studies that examine basic farming methodology. Experiment 5 is a larger, more in-depth study based on experiments 1-4 that explores the most promising methods and materials. Experiment 6 is a preliminary study that examines the commercial potential of two farming structures developed from the previous experiments.

Table 4.1. Experiments done to develop farming structures for the commercial aquaculture of sponges for metabolite production.

Experiment
4.2. Examining farming response to different support materials
4.3. Farming methodology, 1
4.4. Farming methodology, 2
4.5. Examining explant attachment on solid substrates
4.6. Developing farming structures for commercial sponge aquaculture
4.7. Two commercial sponge farming structures

Because these experiments were first attempts at choosing methods and materials, it was anticipated that many would yield poor sponge growth. The evolution of the best

methods and materials will be based on the most promising results from each experimental step. To show this evolution clearly each experiment is examined and discussed individually.

4.1.1. Collecting sponges

For these experiments *Latrunculia brevis* and *Polymastia croceus* were collected from the south coast of Wellington and *Raspailia agminata* from Cape Rodney (Fig 1.2). The sponges were cut using a sharp sterile scalpel into cube-shaped explants. One side covered in intact pinacoderm was not cut while the other five sides showed exposed mesohyl.

Unless otherwise stated, each explant was attached to the substrate with its uncut side facing up. All explants were separated by 5cm in laboratory experiments to prevent chemical interactions, and all dead explants were removed to prevent cross infection.

4.2 Examining farming response to different support materials

4.2.1. Introduction

The importance of using appropriate support material to promote sponge growth and attachment was realised at an early stage. At the start of this century Moore (1908a) found that bath sponges readily attach and grow on concrete but not on wood. With the greater range of materials available today, especially plastic composites, it is important to explore their suitability as support structures. In this experiment *Latrunculia brevis* was grown on a range of materials: glass, polyvinyl chloride (PVC), polypropylene, polyethylene, shell pieces and pebbles. Shell and pebbles could be incorporated into a substrate if they are found to be the most suitable materials. The heavy weight of concrete makes it unsuitable as a substrate for hanging culture and therefore it was not tested.

4.2.2. Methods

In the laboratory, *L. brevis* explants 9g (SE=0.3) in weight were placed onto solid sheets (20x30cm) of glass, PVC, polypropylene and polyethylene, and loose sediment of marine shell and aquarium pebbles. Both marine shell and aquarium pebbles had a particle size of 5mm. Each of the six substrates had 4 replicates, set-up in individual aquaria (one substrate replicate per aquaria). Each substrate had sixteen explants, four per replicate. The aquaria were 50l in volume. The sea-water was sourced from a depth of 16m in

Wellington Harbour, adjacent to the laboratory. The flow rate to each aquarium was ~1l every 10secs.

This experiment ran for 52 days from 24 September 1996 to 15 November 1996.

4.2.3. Results

Explant survival was similar on all six substrates (One-Way ANOVA: $F_{df(5,18)}=0.04$, P=0.99), averaging 80% (Fig. 4.1a). Overall growth was poor with most explants shrinking by 25% of their initial weight (Fig. 4.1b). Material composition did not affect growth (One-Way ANOVA: $F_{df(5,18)}=1.15$, P=0.37). No explants became attached to any of the eight substrates. No necrosis was observed between explants and the substrate in any treatment.

4.2.4. Conclusion

Because weight loss was similar across all six treatments it probably results from a lack of food, and not due to substrate composition. Starvation may also explain why no explants attached to any of the substrates. High survival on all substrates is a promising result. Overall, this experiment suggests that a range of support materials are suitable for farming *L. brevis*.

4.3. Farming methodology, experiment 1

4.3.1. Introduction

Previous studies (Moore 1908a, Crawshay 1939, Verdenal and Vacelet 1990, Battershill and Page 1996, Duckworth et al. 1997, Pronzato et al. 1999) have farmed sponges experimentally using several structures or methods. These can be grouped into three general methods: on substrate pieces, inside mesh, and with wire threaded through them. However, no study so far has examined and directly compared each of these methods simultaneously with each other. Therefore, in this experiment, these methods were tested for growth and survival of *Latrunculia brevis*.

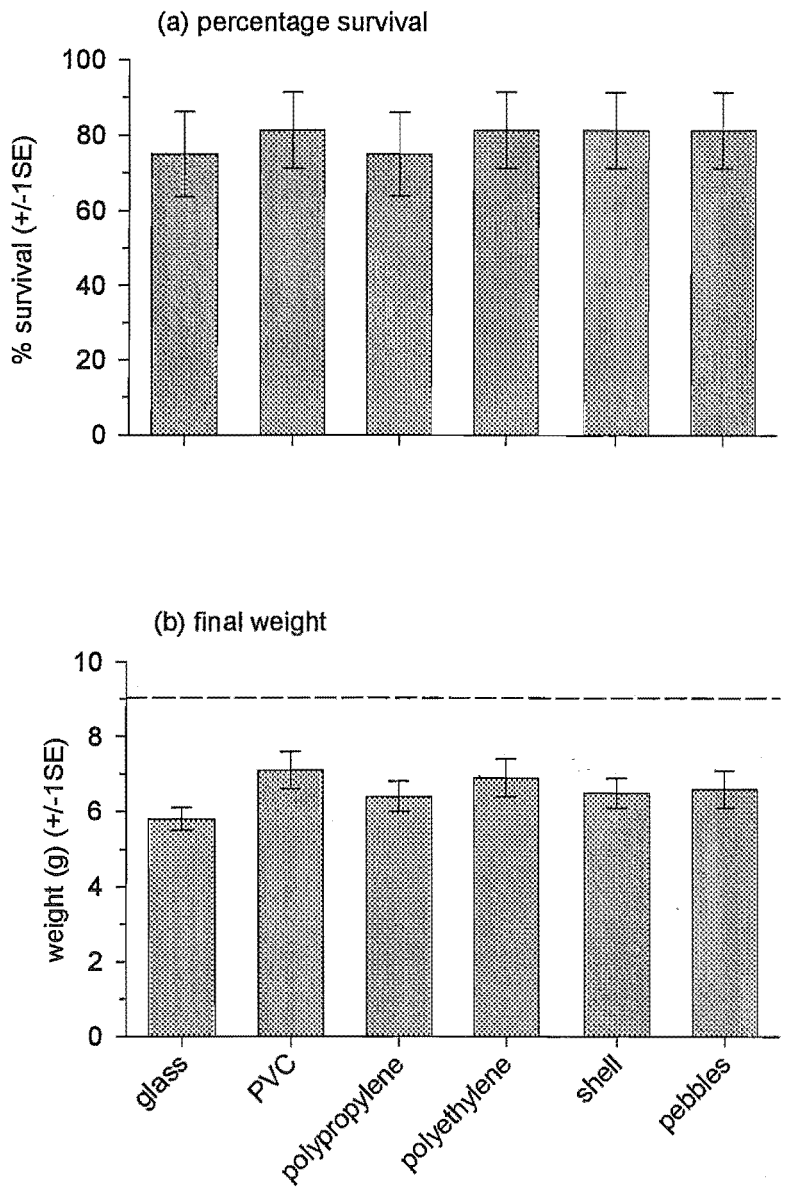


Figure 4.1. Mean percentage survival and final weight of *L. brevis* after 52 days on a range of support materials. Dashed line represents initial explant weight. Error bars represent variation between aquaria for survival and between explants for final weight.

4.3.2. Methods

At Mahanga Bay in Wellington Harbour (Fig. 1.2), *L. brevis* explants of 16g (SE=0.5) were farmed using three general methods: on substrate pieces; inside mesh; and with rope threaded through them (Table 4.2). The first two general methods were further subdivided into several specific methods to test a greater range of materials.

General method 1. Explants were tied and secured with cable-ties to ~40cm^2 pieces of slate, concrete-fibrelight composite, and polypropylene (Fig. 4.2a). Half of the polypropylene pieces had holes, 7mm in diameter, which allowed the explant to grow through the substrate. The surface of the polypropylene pieces were roughened with sandpaper to aid explant attachment. The other two substrates were already rough.

Table 4.2. General and specific methods used to farm explants of *L. brevis* in the first *in situ* experiment.

General method	Specific method
(1) explant farmed on substrate	slate
	concrete-fibrelight composite
	polypropylene, with holes
	polypropylene, without holes
(2) explants farmed inside mesh	scallop lanterns
	individual mesh bags
(3) rope threaded through explant	nylon cable-ties

General method 2. Explants were farmed in scallop lanterns and individual mesh bags (Fig. 4.2b). Both lanterns and mesh bags were made of nylon mesh, with hole sizes of 20mm and 10mm respectively. This difference in hole size was not considered important at this early stage of farming trials. The major and important difference between these two methods was explant separation; in individual mesh bags explants were held separately, while in lanterns they were grouped and held together.

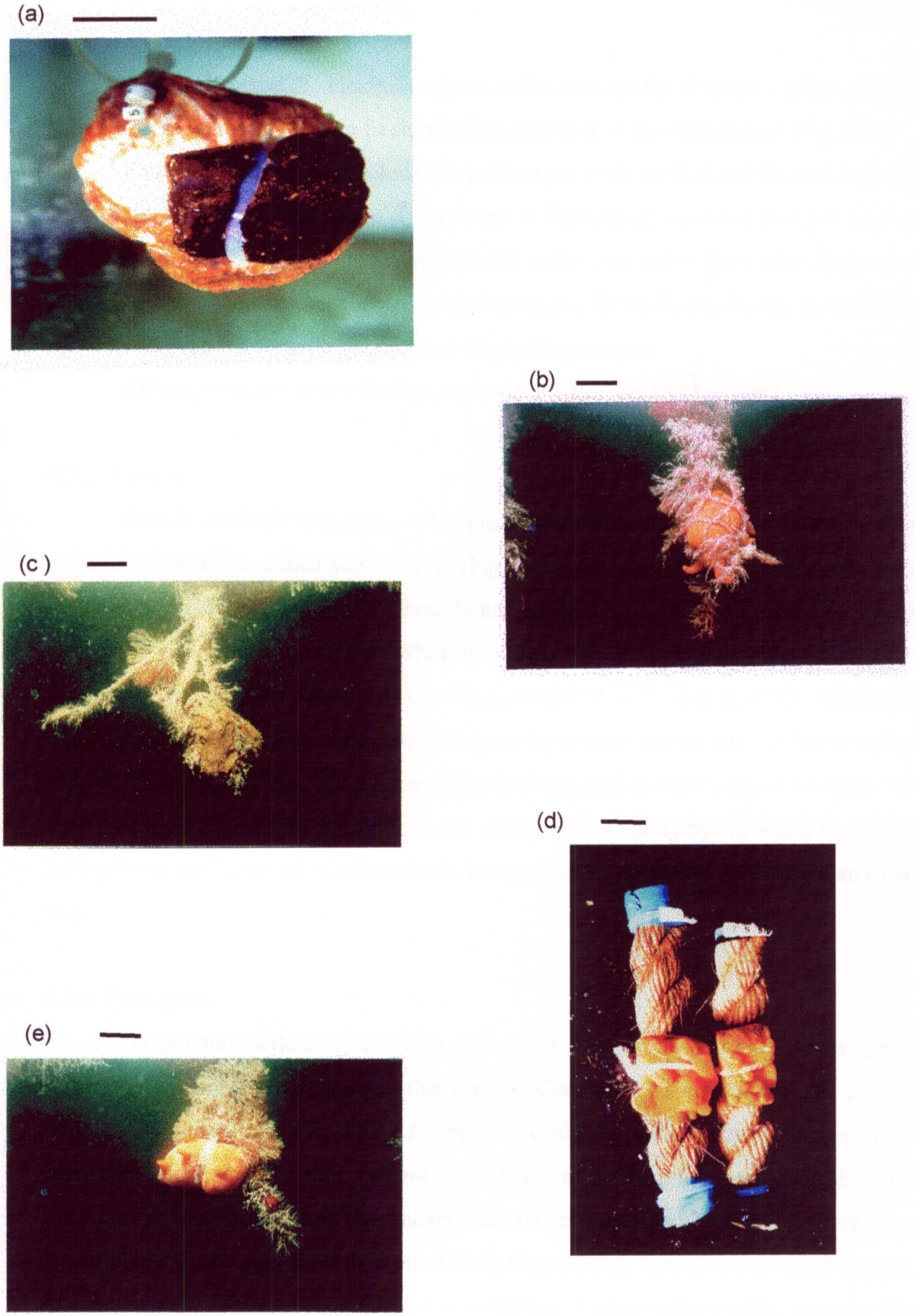


Figure 4.2. Photographs of *R. agminata* (brown), *P. croceus* (orange) and *L. brevis* explants (green) in some of the experimental methods examined to develop a farming structure for commercial sponge aquaculture. Each photograph represents a general method used: explants were farmed (a) on substrate pieces, (b) inside mesh,(c) with threaded rope, (d) on rope and, (e) with rope wrapped around them. Black bars represents 1cm.

General method 3. Finally, explants were secured by threading nylon cable-ties through them so that they hung freely and fully exposed to the environment (Fig. 4.2c)

Fourteen explants were allocated randomly to each method and farmed at a depth of 10m in two areas 20m apart. There were 7 explants to each method per area. All explants, except those farmed in scallop lanterns, were attached at 15cm intervals to a rope back-line, and coded so that their individual progress could be monitored. In the scallop lanterns, all explants were placed within a single compartment.

This experiment ran for 39 days from 4 April 1997 to 13 May 1997.

4.3.3. Results

Overall survival was poor. All explants tied to and farmed on substrate pieces (general method 1) had died after 39 days (Fig. 4.3a). The final survival of explants farmed in lanterns, mesh bags (general method 2) and secured with threaded cable-ties (general method 3) was similar, averaging 48% (Fig. 4.3a). The final weights were similar across the three methods (One-Way ANOVA: $F_{df(2,17)}=0.26$, $P=0.776$) (Fig. 4.3b). While there was an overall weight loss, three explants grew by several grams: two cable-tie explants and one mesh bag explant. All three explants had attached to their support structure. The mesh bag explant was growing through the nylon mesh. Fouling by algae and the hydroid *Aglaophenia* sp. occurred in all methods but was most extensive on lanterns and mesh bags.

4.3.4. Conclusion

The general method of attaching explants of *L. brevis* to substrate pieces appears unsuitable as a method for farming this species. Considering the results of the previous laboratory-based experiment, where *L. brevis* had high survival when placed on various substrates, it is unlikely that explant mortality was caused by toxins leaching from substrates or by abrasion. It is also unlikely that the explants died because substrate pieces drastically reduced water flow. It is more likely that the high mortality in general method 1 was due to tissue damage caused by tying cable-ties around explants when securing them to substrate pieces. Barthel and Theede (1986), studying *Halichondria panicea*, also found greater mortality of explants when tied to substrate as compared to suspended explants. The trade-off is between adequately securing the explant so that it will not float away and causing tissue damage.

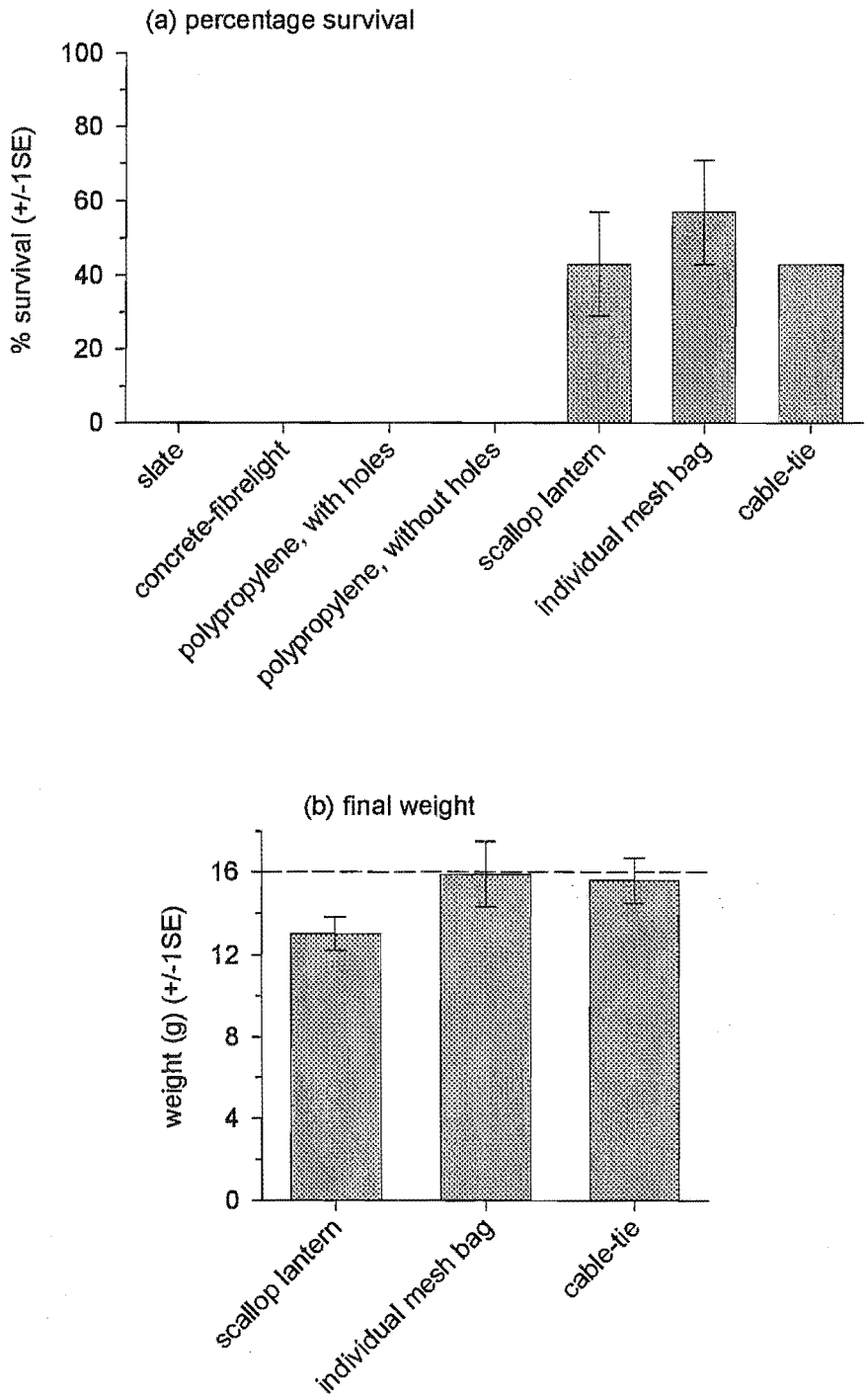


Figure 4.3. Mean percentage survival and final weight of *L. brevis* farmed after 39 days across experimental treatments. Dashed line represents initial explant weight. Error bars represent variation between areas for survival and between explants for final weight.

Both scallop lanterns and mesh bags show some promise as methods for farming *L. brevis*. However, high levels of biofouling reducing water movement to explants was a problem. Because fouling varies from site to site (Claereboudt et al. 1994), careful site selection can reduce fouling and improve explant growth and survival. The growth in mesh bags was promising and could lead to farming structures not seen before in sponge aquaculture. Both lanterns and individual mesh bags merit further study.

Farming *L. brevis* by threading cable-ties through explants also shows promise. It is possible this method will promote higher explant growth than lanterns and mesh bags because explants are fully exposed to the environment and have less biofouling than other methods.

4.4. Farming methodology, experiment 2

4.4.1. Introduction

Based on the results from the previous experiment some details of farming methods were changed for further testing. Two new substrate treatments, "oyster shell" and "polyethylene" were added and the substrate treatment "polypropylene with holes" was removed. Thus a greater range of natural and artificial substrates was examined. To investigate further the method of passing rope through explants a polyvinyl alcohol (PVA) rope treatment was included. As in the previous experiment, these treatments could be grouped into three categories: explants farmed on substrate pieces; explants farmed inside mesh; and rope threaded through explants. This experiment examined the farming response of the sponge *Raspailia agminata*.

4.4.2. Methods

At Mahanga Bay, *R. agminata* explants of 18g (SE=0.8) were farmed using three general methods: on substrate pieces, inside mesh, and with rope threaded through them (Table 4.3). Each general method was subdivided into several specific methods.

General method 1. Explants were tied and secured with cable-ties to ~40cm^2 pieces of slate, oyster shell, concrete-fibrelight composite, polypropylene and polyethylene (Fig. 4.2a). Using sandpaper the surface of polypropylene and polyethylene pieces were roughened as a possible aid to explant attachment.

General method 2. Explants were grouped and farmed together in scallop lanterns, or were farmed separately in individual mesh bags (Fig. 4.2b). Both lanterns and mesh bags were made of nylon mesh, with hole sizes of 20mm and 10mm respectively.

Table 4.3. General and specific methods used to farm explants of *R. agminata* in the second *in situ* experiment.

General method	Specific method
(1) explant farmed on substrate	slate
	oyster shell
	concrete-fibrelight composite
	polypropylene
	polyethylene
(2) explants farmed inside mesh	scallop lanterns
	individual mesh bags
(3) rope threaded through explant	nylon cable-ties
	PVA rope

General method 3. Finally, explants were secured by threading nylon cable-ties or PVA rope through them so that they hung freely and fully exposed to the environment (Fig. 4.2c).

Fourteen explants were allocated randomly to each specific method and farmed at a depth of 10m in two areas 20m apart. There were 7 explants to each method per area. All explants, except those farmed in lanterns, were attached at 15cm intervals to a rope back-line, and coded so that their individual progress could be monitored. In the scallop lanterns, all explants were placed within a single compartment.

This experiment ran for 25 days from 18 April 1997 to 13 May 1997.

4.4.3. Results

Overall survival was very poor with only 17 explants surviving from the 126 transplanted. Survival was greatest at about 50% in the PVA rope method (Fig. 4.4a). There was a significant difference in growth between the five methods (One-Way ANOVA: $F_{df(4,11)}=9.46$, $P=0.001$). Explants farmed in mesh bags lost the least weight (Fig.

4.4b). No explants attached to any of the solid substrate treatments. However, two of the seven surviving explants threaded with PVA rope became attached to the rope. Unlike *L. brevis* in the previous experiment, these explants lost weight.

4.4.4. Conclusion

Only the mesh bag and PVA rope methods showed promise as suitable methods, but no method worked well. Two factors may have contributed to the weight loss of *R. agminata* in this experiment. Explants were farmed experimentally in Wellington Harbour (14°C) which is colder than Cape Rodney (18°C) where they were collected. Cool water can arrest explant growth. Simpson (1968) discovered that explants of *Microcinia prolifera* did not grow in seawater below 15°C. Barthel and Theede (1986) found that lowering water temperature below 5°C stops explant growth of *Halichondria panicea*. In addition, the results of a reciprocal transplant experiment (Chapter 3) suggested that growth of relocated *P. croceus* explants was retarded until they adjusted to their new environment. In this experiment, *R. agminata* was transplanted ~700km away from their source location and farmed for 25 days which may have been insufficient time for explants to adjust to their new environment and to have grown. These factors may have also increased mortality of *R. agminata* explants.

Although explant health was compromised, this experiment suggested that two methods, individual mesh bags and threaded PVA rope, had potential for farming *R. agminata*. Once again, farming explants on substrate pieces (general method 1) produced the worst results. Overall survival on substrate was 3% (2/70). In the previous experiment, *L. brevis* seemed to be easily damaged when tied to substrate pieces. Considering this finding, squeezing or damaging *R. agminata* explants when tying them onto substrate was avoided. Unfortunately, explant survival was still poor. The farming of explants on substrate pieces may have limited commercial potential for these species.

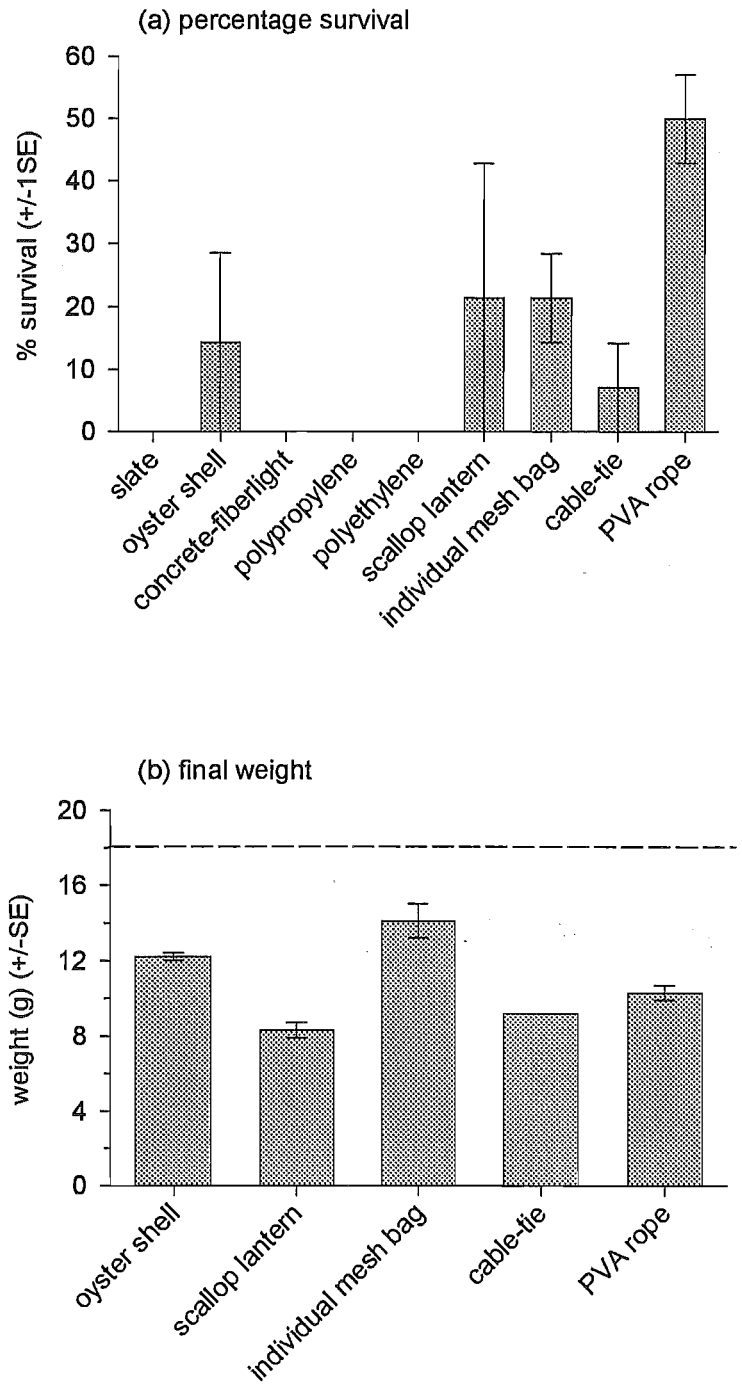


Figure 4.4. Mean percentage survival and final weight of *R. agminata* after 25 days across experimental treatments. Dashed line represents initial explant weight. Error bars represent variation between areas for survival and between explants for final weight.

4.5. Examining explant attachment on solid substrate

4.5.1. Introduction

The finding that no explant of either *Latrunculia brevis* or *Raspailia agminata* attached to any of the tested substrates in the 3 earlier experiments is surprising, considering that previous studies have found other sponge species readily attach to a great range of natural and artificial materials, including concrete, lead, rubber (Moore 1908a), glass (Barthel and Theede 1986), shell, stone (Rosell and Uriz 1992) and plastic (Wilkinson and Vacelet 1979). One possible reason for this lack of attachment is explant orientation. Both Moore (1908a) and Crawshay (1939) found that commercial bath sponges attach quicker if their uncut side, covered in pinacoderm, is against the substrate. The importance of explant orientation for *L. brevis, R. agminata* and *Polymastia croceus* using slate and oyster shell was examined. A previous study (Battershill and Bergquist 1990) found that buds of *P. croceus* attach to rock and shell pieces.

4.5.2. Methods

Twelve explants each of *L. brevis, R. agminata* and *P. croceus* were placed, without tying down, onto ~40cm^2 pieces of slate and oyster shell, one explant per piece. For all three species, each substrate type had three explants with the pinacoderm covered side against the substrate, and three explants with the pinacoderm facing up. The treatments for each species were distributed randomly to two aquaria. An aquarium food block (Reefclear, Aquarium Pharmaceuticals), specially designed for feeding marine invertebrates, was placed into each tank to promote explant growth. The aquaria were 50l in volume, with the sea-water sourced from a depth of 16m in Wellington Harbour. The flow rate to each aquaria was ~1l every 10secs.

The experiment ran for 29 days from 6 June 1997 to 5 July 1997.

4.5.3. Results

Regardless of orientation, no explant of *L. brevis, R. agminata* and *P. croceus* attached to slate or shell. For each species, healing of cut sides was similar between all treatments. All explants survived and most had stable weight.

4.5.4. Conclusion

In previous experiments explants of *L. brevis* and *R. agminata* were observed to attach to their support structure within 4 weeks. Barthel (1986) recorded that *Halichondria panicea* can attach to glass in 3 days. Therefore it was considered that the explants had sufficient time to heal and attach. This experiment therefore suggests that explant orientation, or whether the side against the substrate is covered with pinacoderm or not, is unimportant for the attachment of *L. brevis*, *R. agminata* and *P. croceus* explants to solid substrate. The reason why no explants attached to pieces of rock and shell, substrates which they are naturally found on (Battershill and Bergquist 1990, Duckworth 1994; Chapter 2), is unknown.

4.6. Developing farming structures for commercial sponge aquaculture

4.6.1. Introduction

Sponge growth and survival are clearly affected by the method of farming. The next step is a larger, more in-depth experiment exploring specific characteristics of each method viewed as having commercial potential. For example, when farming sponges in mesh should they be grown separately or together, and what mesh size is best? Or, when farming sponges with rope threaded through them should rope of natural or artificial origin be used? To determine which method is most suitable to commercially farm sponges, it is also important to compare explant bioactivity along with growth and survival. Measuring explant bioactivity will indicate how farming methodology may affect the production of the target bioactive metabolite.

In this experiment *Latrunculia brevis*, *Polymastia croceus* and *Raspailia agminata* were farmed using four general methods: explants farmed inside mesh; explants farmed on rope; rope threaded through explants; and rope wrapped around explants. Although no explants attached to substrate pieces (e.g. slate, shell, plastic) in previous experiments, it was considered important to farm explants on rope-substrate in this experiment to compare a wide range of possible methods. Each general method of farming was divided into several specific methods examining the importance of mesh size or rope composition.

4.6.2. Methods

The four general methods and numerous specific methods were tested simultaneously (Table 4.4). In general method 1, lanterns and individual mesh bags were

examined. These differ in the degree of explant separation; explants in lanterns were grouped and farmed together in a single compartment, while explants in mesh bags were farmed separately. The effect of mesh size and strand thickness was also examined as both factors may affect water flow (Table 4.5).

In the other three general methods a range of natural and artificial ropes were trialed to test the importance of rope material. The ropes tested within each general method all had similar thickness, but varied in texture (Table 4.6). All natural fibre ropes were free of artificial compounds. In general method 2, each explant was secured to a separate 15cm length of rope with cotton (Fig. 4.2d). In general method 4, the rope or cable-tie was secured around each explant with sufficient pressure to contain the explant but without damaging it (Fig. 4.2e)

Table 4.4. General and specific methods tested on *L. brevis*, *P. croceus* and *R. agminata* in the large farming experiment.

General method	Specific method	Mesh characteristics or rope material
1, explant farmed	scallop lanterns	explants farmed together; large mesh
inside mesh	pearl lanterns	explants farmed together; fine mesh
	ind. mesh bags - a	explants farmed separately; fine mesh, thin strand
	ind. mesh bags - b	explants farmed separately; large mesh, thin strand
	ind. mesh bags - c	explants farmed separately; large mesh, thick strand
2, explant farmed	manila	natural rope
on rope	sizel	natural rope
	nylon	artificial rope
	polypropylene	artificial rope
3, rope threaded	cotton	natural rope
through explant	hemp	natural rope
	PVA	artificial rope
	cable-tie	artificial rope
4, rope wrapped	cotton	natural rope
around explant	cable-tie	artificial rope

Table 4.5. Mesh characteristics of the five mesh methods in general method 1. Mesh cover represents the percent area covered by mesh, determined by digitising a sample and using the computer programme OPTIMAS to calculate its percent area. Each percentage is the average of 5 mesh samples.

Specific method	Mesh size (mm)	Strand thickness (mm)	Mesh cover (%)
scallop lantern	30	1	10
pearl lantern	10	1	18
individual mesh bag-a	10	1	17
individual mesh bag-b	30	1	11
individual mesh bag-c	30	2.5	23

Table 4.6. Characteristics of rope types used in general methods 2, 3 and 4.

General method	Specific method	Width (mm)	Texture
2, explant farmed	manila	24	hard, hairy
on rope	sizel	24	hard, hairy
	nylon	24	soft, smooth
	polypropylene	24	hard, smooth
3, rope threaded	cotton	2.5	soft, hairy
through explant	hemp	3	hard, smooth
	PVA	3	soft, hairy
	cable-tie	3	hard, smooth one side
4, rope wrapped	cotton	2.5	hard, hairy
around explant	cable-tie	3	hard, smooth one side

Ten explants or replicates were allocated randomly to each specific method. These were transplanted onto two arrays situated 15m apart, five explants per method per array. All explants, except those placed in lanterns, were individually labelled and farmed separately at 15cm intervals along the array. An array consisted of a rope back-line situated at a depth of 10m at Mahanga Bay.

The initial explant weight between specific methods was not significantly different for either *L. brevis*, *P. croceus* or *R. agminata* (Table 4.7). This experiment ran from mid

October 1997 to late January 1998. The experiment time was 85 days for *L. brevis*, 87 days for *P. croceus*, and 103 days for *R. agminata*. The times differed slightly because only one species could be prepared at a time.

Table 4.7. Mean initial weight (±1SE) for *L. brevis*, *P. croceus* and *R. agminata* across all treatments. For each species, One-Way ANOVAs examined initial explant weight variability between specific methods. DF=14,35 for each species. Prob: *=significant.

Species	$\bar{x}$ (±1SE)	F-ratio	Prob
L. brevis	14.8 (0.3)	1.11	0.35
P. croceus	15.8 (0.2)	1.59	0.09
R. agminata	18 (0.3)	1.65	0.08

At the end of the experiment, explants of *L. brevis* and *P. croceus* were analysed chemically against a P388 murine leukaemia bioassay to determine if the method of farming affected explant bioactivity. Two grams of explant tissue from each specific method, consisting of 0.4g sub-samples cut from 5 randomly chosen explants, were analysed. Because of the costs involved, it was not possible to analyse several replicate explants per method individually. However, previous analysis has indicated that the bioassay method generates precise bioactivity scores for each species (Table 3.4&6). In addition, five explants of *L. brevis* and *P. croceus* from one specific method were analysed individually to examine variability within a treatment. The sampling procedure is described in Chapter 2. *R. agminata* was not assayed because it grew poorly in this experiment.

4.6.3. Results

In addition to the full ANOVA model, one-way ANOVA's examined whether explant growth of each species was affected by rope material (natural vs. artificial) in general methods 2 and 3, and explant separation (lanterns vs. individual mesh bags) in general method 1. A method was considered to have promoted good growth if mean final explant weight was >110% of initial weight.

4.6.3.1. *Latrunculia brevis*

The final weights differed greatly between the methods tested (Table 4.8a). Final weights were greatest for explants farmed in scallop lanterns, individual mesh bags-b, and with threaded PVA rope (Fig. 4.5a). Final weights were similar between explants farmed inside lanterns and mesh bags ($F_{df(1,3)}$=0.09, P=0.789). Over half of the explants farmed in mesh bags-a and -b (both made of thin strand) grew partially through the mesh. Final weights were also similar between explants threaded with natural fibre and artificial rope ($F_{df(1,2)}$=0.15, P=0.739). Six of the 10 explants farmed with threaded PVA rope had attached to the rope by the end of the experiment.

Table 4.8. Analysis of variance for final weight and survival of *L. brevis* explants in the large farming experiment. GLM ANOVA used to analyse data. To meet assumptions, survival data were arcsine transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
(a) final weight					
general method	3	145.5	48.5	0.58	0.646
specific method (general)	8	673	84.1	5.99	<0.0001*
error	79	1109.5	14		
total	90	1928			
(b) survival					
general method	3	8.115	2.705	9.78	0.002*
specific method (general)	11	3.043	0.277	4.9	0.002*
error	15	0.847	0.056		
total (adjusted)	29	12.005			

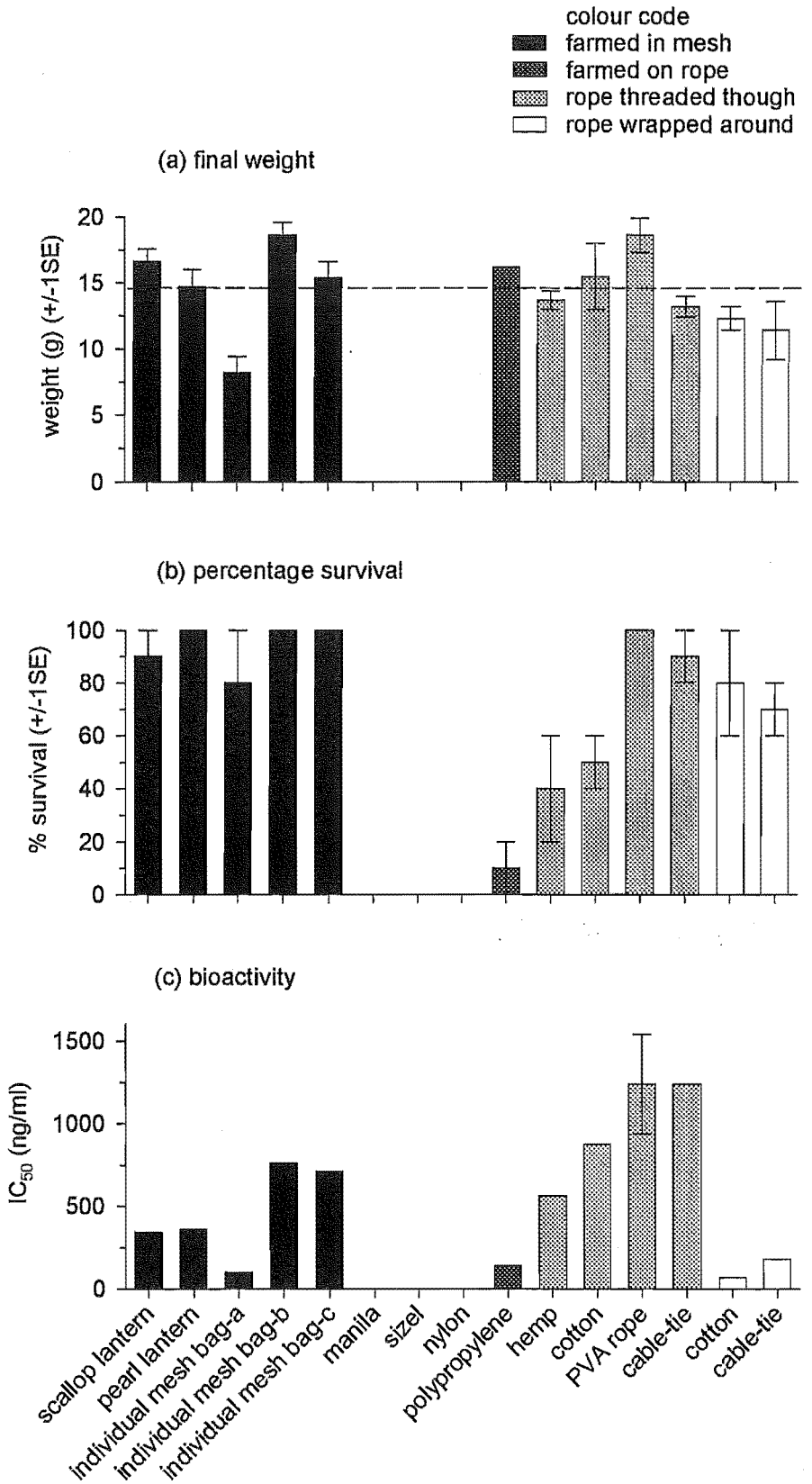


Figure 4.5. Mean final weight, percentage survival and bioactivity of *L. brevis* between specific farming methods after 85 days. Dashed line represents initial explant weight. Error bars represent variation between explants for final weight, between arrays for survival, and between 5 explants with threaded PVA rope for bioactivity. As IC_{50} decreases, bioactivity increases.

Survival of *L. brevis* also differed greatly between methods (Table 4.8b). Survival was greatest (100%) for explants farmed in pearl lanterns, individual mesh bags-b and -c, and with threaded PVA rope (Fig. 4.5b). Comparing general methods, survival was greatest for explants farmed in mesh (94% survived) and lowest for explants farmed on rope (general method 2) (3%; 1/40 survived). Many of the explants farmed on rope grew away from the rope and tie, subsequently dislodging themselves. The one remaining explant did not attach to its rope support.

Explant bioactivity was also affected by the method of farming (Fig. 4.5c). The greatest bioactivity (<200ng/ml) was in the explants farmed in mesh bag-a, on polypropylene rope, and with cotton rope and cable-tie wrapped around them. Correlation analysis between specific methods showed an inverse relationship between bioactivity and final weight (Canonical correlation: r=0.583, P=0.047). Therefore, bioactivity was generally low in the methods where growth was greatest, such as mesh bag-b and threaded PVA rope (Fig. 4.5a,c). Variation between explants within a method was low (Table 4.9a). Therefore, the differences in sponge bioactivity may represent real treatment effects.

Table 4.9. Mean ($\bar{x}$), standard error (SE) and coefficient of variation (CV) of bioactivity (ng/ml) between 5 explants of (a) *L. brevis* and (b) *P. croceus* farmed on threaded PVA rope.

	$\bar{x}$	SE	CV
(a) *L. brevis*	1144	300	26
(b) *P. croceus*	357	146	41

4.6.3.2. *Polymastia croceus*

The final weights differed greatly between the methods tested (Table 4.10a). Final weights were greatest for explants farmed in scallop lanterns and mesh bag-b (Fig. 4.6a). Final weights were similar between explants farmed inside lanterns and mesh bags ($F_{df(1,3)}$=0.02, P=0.896). By the end of the experiment, 3 explants farmed in mesh bags-a and 2 explants in mesh bags-b had grown partially through the mesh. Rope material

(natural vs. artificial) did not affect the final weights of explants in general method 2 ($F_{df(1,2)}$=0.12, P=0.76) or general method 3 ($F_{df(1,2)}$=5.64, P=0.141). Apart from 9 explants farmed with threaded PVA rope no explants attached to their rope support.

Survival was similar between specific methods but differed greatly between general methods (Table 4.10b). Survival was greatest for explants farmed inside mesh (100%) and with threaded rope (98%) (Fig. 4.6b). Poor survival of explants farmed with cable-ties wrapped around them (Fig. 4.6b) resulted from explant rejection of the support material.

Explant bioactivity of *P. croceus* varied greatly between specific methods (Fig 4.6c). Generally, bioactivity was high for explants grown on artificial material such as PVA rope, and low for explants grown on natural materials such as cotton. Between specific methods, explant bioactivity was not correlated with average final weight (Canonical correlation: r=0.178, P=0.526). Explants of *P. croceus* had greater variability in bioactivity within a method than explants of *L. brevis* (Table 4.9b).

Table 4.10. Analysis of variance for final weight and survival of *P. croceus* explants in the large farming experiment. GLM ANOVA used to analyse data. To meet assumptions, weight data were log transformed, and survival data were arcsine transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
(a) final weight					
general method	3	0.066	0.022	0.48	0.7
specific method (general)	10	0.498	0.045	5.19	<0.0001*
error	115	1.103	0.009		
total	129	1.67			
(b) survival					
general method	3	3.242	1.081	8.13	0.004*
specific method (general)	11	1.461	0.132	1.1	0.421
error	15	1.808	0.121		
total	29	6.511			

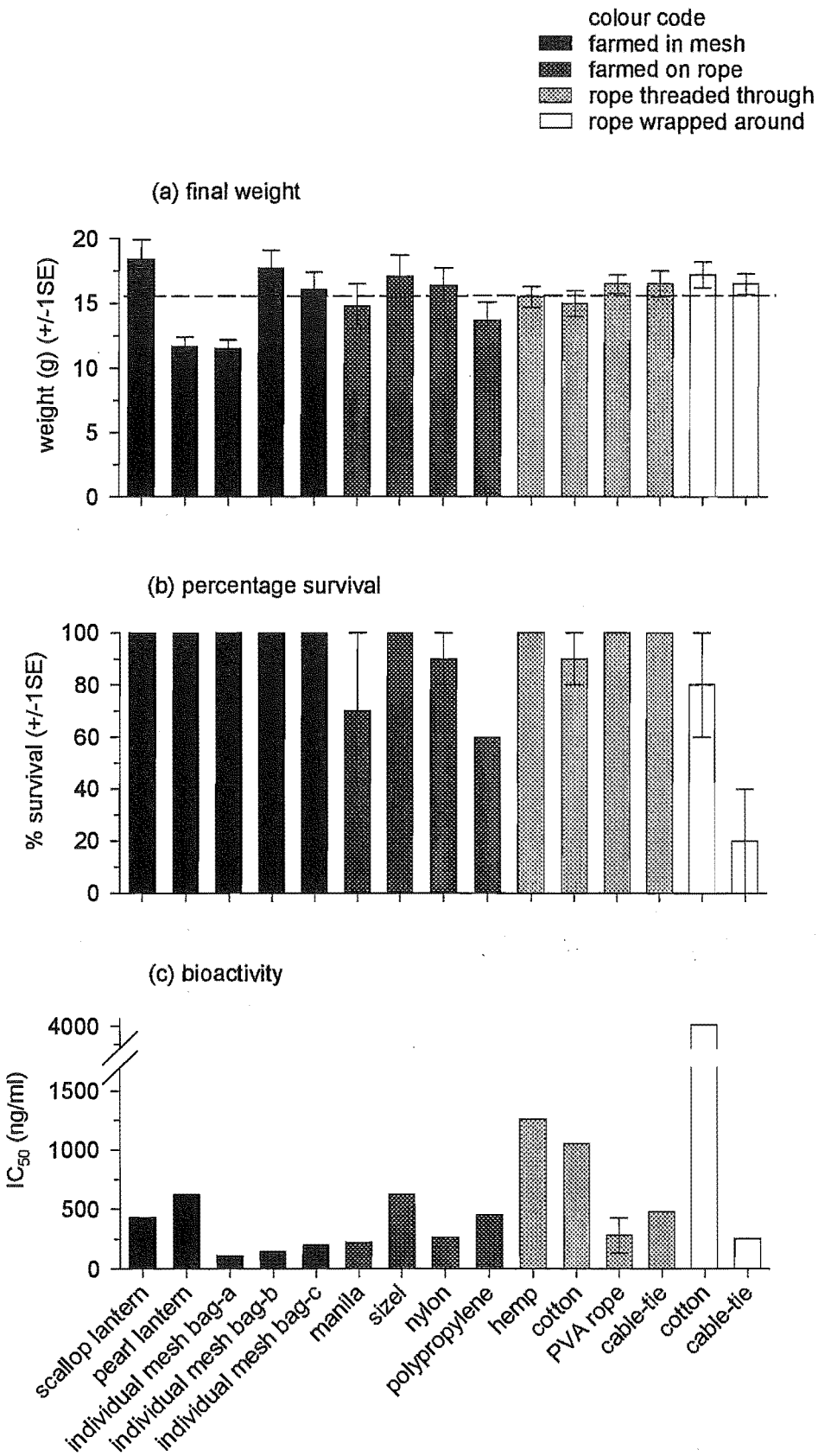


Figure 4.6. Mean final weight, percentage survival and bioactivity of *P. croceus* between specific farming methods after 87days. Dashed line represents initial explant weight. Error bars represent variation between explants for final weight, between arrays for survival, and between 5 explants with threaded PVA rope for bioactivity. As IC_{50} decreases, activity increases.

4.6.3.3. *Raspailia agminata*

The final weights were similar between all specific and general methods (Table 4.11a, Fig. 4.7a). Overall growth was poor with >75% of explants losing weight. Final weights were similar between explants farmed inside lanterns and mesh bags ($F_{df(1,3)}$=0.75, P=0.45). One explant farmed in mesh bag-b had grown partially through the mesh after 2 months. Rope material (natural vs. artificial) did not influence the final weights of explants in general method 2 ($F_{df(1,2)}$=1.32, P=0.37) or in general method 3 ($F_{df(1,2)}$=0.00, P=0.983). Apart from 8 explants farmed with threaded PVA rope no explants attached to their rope support.

Survival differed between general methods (Table 4.11b) and was greatest for explants farmed inside mesh (98% overall) (Fig. 4.7b). As for *L. brevis* and *P. croceus*, all explants of *R. agminata* farmed with threaded PVA survived (Fig. 4.7b).

Table 4.11. Analysis of variance for final weight and survival of *R. agminata* explants in the large farming experiment. GLM ANOVA used to analyse data. To meet assumptions, weight data were log transformed, and survival data were arcsine transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
(a) final weight					
general method	3	0.114	0.038	2.98	0.078
specific method (general)	11	0.014	0.013	1.16	0.322
error	111	1.222	0.011		
total (adjusted)	125	1.477			
(b) survival					
general method	3	2.39	0.797	8.91	0.003*
specific method (general)	11	0.983	0.089	0.82	0.625
error	15	1.634	0.109		
total (adjusted)	29	5.01			

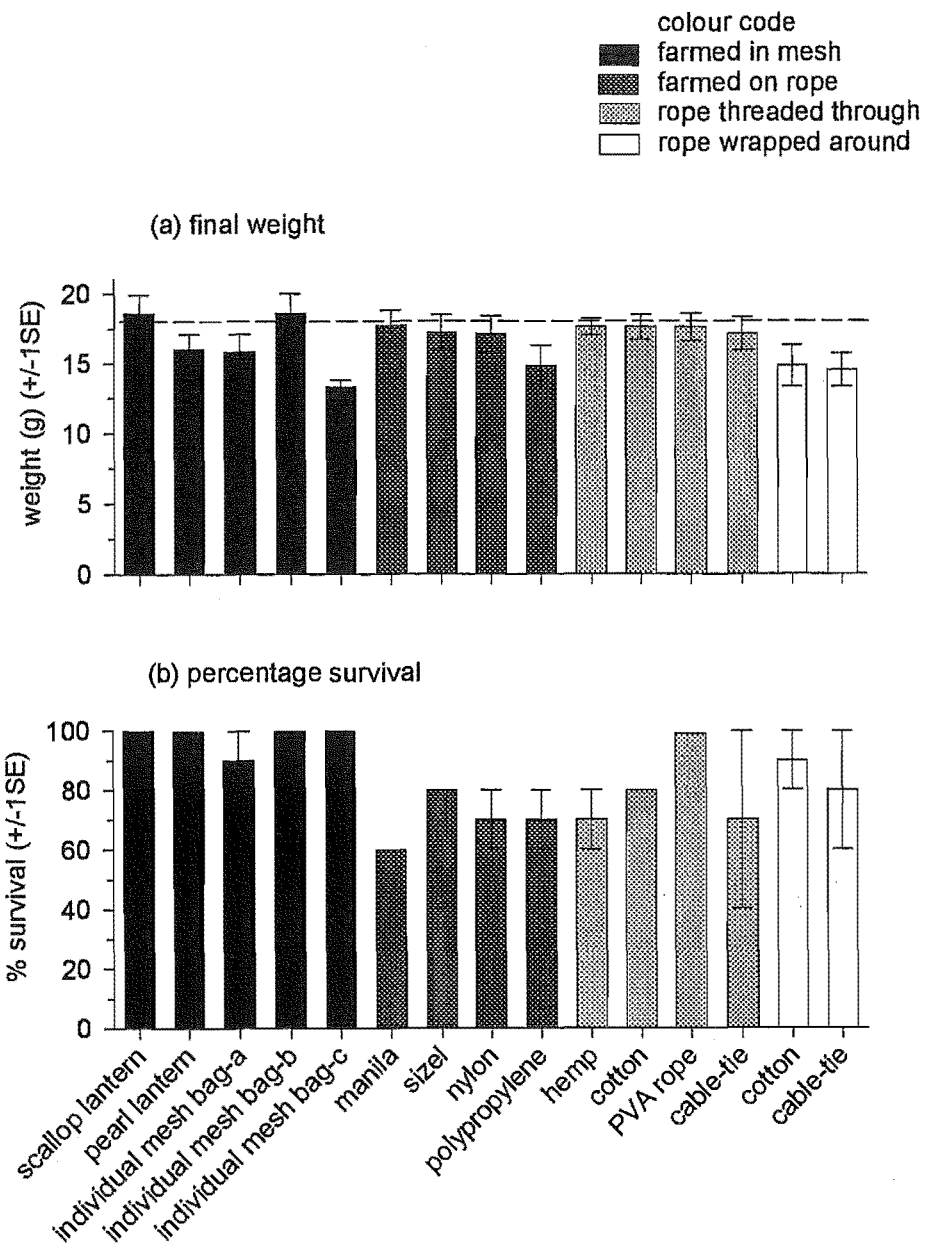


Figure 4.7. Mean final weight and percentage survival of *R. agminata* between specific farming methods after 103 days. Dashed line represents initial explant weight. Error bars represent variation between explants for final weight and between arrays for survival.

4.6.4. Conclusion

The method of farming clearly had an effect on the growth, survival and bioactivity of sponges. For *L. brevis*, *P. croceus* and *R. agminata*, survival was greatest for explants farmed in mesh and lowest for explants attached to and farmed on rope, or with rope wrapped around them. Good survival of explants farmed in mesh is due mainly to two factors. Most importantly, explants in this method experience the least initial damage, as they are simply placed into mesh. Explants in the other general methods have either rope pushed through or squeezed around them. This causes tissue damage and increases the chance of mortality. Second, even if the mesh method is not ideal the explant is effectively trapped. Many explants in the other three general methods moved or grew away from the rope until they eventually dislodged themselves. The rejection by *R. agminata* explants of threaded cable-ties (Duckworth et al. 1997) prompted the first trial of individual mesh bags for farming sponges.

Explant rejection of the rope reduces survival and is unacceptable for commercial production. The one promising result from these rope methods was the high attachment of explants with PVA rope threaded through them. In this method, all explants from all three species survived. In addition, *L. brevis* farmed with threaded PVA rope grew well, with an average growth of 3g or 20% of initial weight per explant. Attachment to the support structure can be important. Verdenal and Vacelet (1990) discovered that explants of *Spongia officinalis* that adhered to their fastening wire or identification tag had the highest growth rates.

The good attachment of *L. brevis*, *P. croceus* and *R. agminata* to threaded PVA rope probably results from the rope's physical and chemical nature. PVA rope has a hairy texture which may promote the secure attachment of explants. In addition, PVA rope does not rot in seawater, unlike ropes made of natural fibres. Rotting of the threaded cotton rope, which had a hairy texture similar to PVA rope (Table 4.6), may have prevented explant attachment. Moore (1908a) also found natural fibre ropes to be unsuitable for farming sponges because they rotted and eventually disintegrated in seawater, causing the loss of all explants.

Some explants of *L. brevis*, *P. croceus* and *R. agminata* farmed in individual mesh bags attached to the mesh, incorporating the nylon strands into their tissue. Attachment only occurred, however, for explants farmed in mesh bags made of thin strand (1mm), and not for explants farmed inside thicker mesh (2.5mm), thus indicating that strand thickness

influences the growth of sponges through mesh. Mesh size can also influence sponge growth. Overall growth was greatest in general method 1 for explants farmed in two treatments, scallop lanterns and mesh bags-b, which had a large mesh size. These results indicate that mesh of large size with thin strand, and therefore of low mesh cover (Table 4.5), is desirable for farming sponges because it allows a high water flow to the explants and thereby promotes their growth.

Good growth of sponges farmed inside mesh has also been reported in other studies. Duckworth et al. (1997) recorded several *R. agminata* explants secured in nylon mesh bags doubling in weight after 262 days of farming. Battershill and Page (1996) reported a monthly growth rate of up to 5000% for *Lissodendoryx* n. sp. farmed in scallop lanterns. However, one major problem of using mesh to farm sponges is biofouling. Fouling organisms, such as seaweeds and bushy bryozoans (*Bugula* spp.), were observed in this study to settle and grow on the mesh strands and, if not removed, would have reduced water flow to the explants. Removing such biofouling from farming structures is an expensive and labour-intensive job (Hodson et al. 1997). The problem of biofouling was more severe on lanterns than on mesh bags, probably because the larger size of lanterns provides more surface area for the attachment of fouling organisms. Lanterns are also difficult to work with because they are cumbersome. These negative factors probably make lanterns unsuitable for farming sponges commercially.

The good growth of *R. agminata* reported by Duckworth et al. (1997) was not reproduced in this study. Poor growth may have resulted from differences in water temperature between the source and farming site, or possibly the farming period of 3½ months was insufficient time for the explants to adjust fully to their new environment. These factors may have also masked any significant differences in growth of *R. agminata* between the farming methods.

For *L. brevis*, farming methods that restricted growth seemed to promote explant bioactivity. This results either from retention of the bioactive metabolites as explants shrink, or biosynthesis of metabolites in response to the unfavourable farming method. Bioactivity of *P. croceus* also varied between farming methods, generally it was most active when farmed with artificial materials, indicating an aggressive response to the chemicals in the materials. If the biosynthesis of metabolites by *L. brevis* or *P. croceus* is in direct response to the farming method then it may indicate an optimal defence. The optimal defence theory, first suggested for plants (Rhoades 1979) and later for marine sessile

invertebrates such as sponges (Turon et al. 1996), proposes that because of an associated cost the organism will only produce a defence, such as bioactive metabolites, when it is required. In the present study, this may have been in response to the substrate material used. Increased metabolite biosynthesis may also result from competitive interactions (Becerro et al. 1997b), predator attack (Walker et al. 1985), or in response to high light exposure to prevent surface-fouling of algae (Thompson et al. 1987, Kreuter et al. 1992). The bioactivity results in this study suggest that if either *L. brevis* or *P. croceus* were commercially farmed then the yield of the target metabolite would vary depending on the farming method used. This can only be fully examined, however, when the target metabolite has been isolated and determined. For *L. brevis*, the target metabolite may be one of the 15 discorhabdins which have so far been isolated (Northcote, Victoria University of Wellington, personal communication). For *P. croceus*, the target metabolite has not been formally identified.

Of the 15 specific methods examined in this experiment, two methods which showed the most promise were threaded PVA rope, and individual mesh bags with large mesh and thin strand. These two methods now need developing into farming structures suitable for the large-scale commercial aquaculture of sponges for metabolite production.

4.7. Two commercial sponge farming structures

4.7.1. Introduction

To be suitable for large-scale commercial use a farming structure must be inexpensive, have a low surface area to reduce drag and bio-fouling, and allow cost-effective and efficient harvesting. It must also promote good growth and survival while maintaining high metabolite production. Considering these requirements, two methods showing the most farming potential are threaded PVA rope, and individual mesh bags with thin strands. These were developed into rope and mesh arrays.

A rope array consisted of a 2.5m length of PVA rope, 3mm thick, threaded with explants (Fig. 4.8a). Explants were threaded onto rope using a technique developed by Moore (1908a). This involved inserting a rope end into a hollow needle which was then carefully pushed through each explant pulling the rope with it. Once the rope was threaded, a knot was tied below each explant, at 10cm intervals, to prevent it falling on the explant below. The rope array was similar to that used by Moore (1908a) and Crawshay (1939) who farmed sponges experimentally by threading wire through them. More

recently, this method was adapted by Pronzato et al. (1999) who used threaded nylon line to grow several Mediterranean sponge species successfully.

A mesh array consisted of a mesh tube 2m long and 10cm wide, divided into two by a central rope 2.5m in length (Fig. 4.8b). Mesh arrays were made from the same mesh used to make individual mesh bags-b, hence mesh cover was a low 11% (Table 4.5). Pockets were made in the mesh by tying the two mesh sides together with cable-ties at 10cm intervals in a zig-zag pattern down the mesh tube. Explants were carefully pushed down until they rested at the bottom of the mesh pocket. A cable-tie tied above the explant prevented it moving upwards and escaping. This method has never been used before to farm sponges or other marine organisms.

4.7.2. Methods

In February `98 three rope and three mesh arrays were transplanted to a depth of 15m at Barrett Reef (Fig. 1.2). Each array contained ten explants each of *L. brevis* and *P. croceus*. The mean initial explant weight was 16.2g (SE=0.8) for *L. brevis* and 17.9g (0.5) for *P. croceus*. In April `98 (after 61 days), all arrays were removed, and explant survival and weight were recorded.

4.7.3. Results

For *P. croceus*, survival was high on both rope (73%) and mesh (77%) arrays. Final weight was also similar between the two farming methods (One-Way ANOVA: $F_{df(1,43)}=2.05$, P=0.159), averaging 88% of initial weight. However, 13 of the 45 surviving explants gained weight. Most *P. croceus* explants farmed on rope arrays attached to the threaded rope. All *L. brevis* farmed in the rope and mesh arrays died.

4.7.4. Conclusion

The poor growth of *P. croceus* and loss of all *L. brevis* probably resulted from the toxic properties of the dinoflagellate *Gymnodinium brevisulcatum* (Chang 1999) which bloomed in Wellington Harbour shortly after explants were transplanted. Although results were poor, both mesh and rope arrays merit further study to further examine their commercial potential for farming sponges.

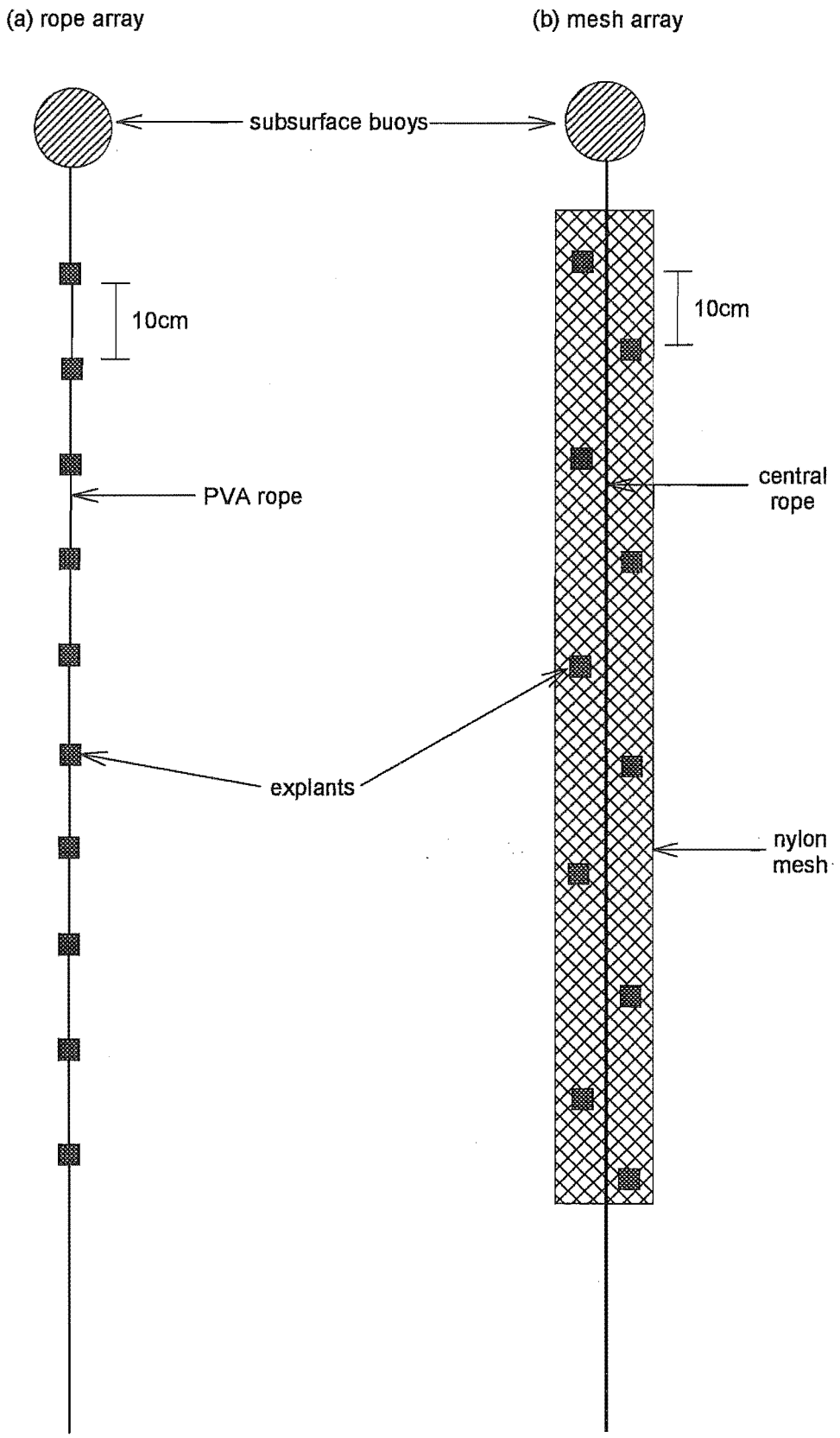


Figure 4.8. Schematic diagram of the rope and mesh arrays used to farm sponges. Each array is 2.5m in length.

4.8. Discussion

An interesting result from this series of experiments was the failure of most explants of *Latrunculia brevis*, *Polymastia croceus* and *Raspailia agminata* to attach to a farming structure (Table 4.12). Explants of these species attached to only four of the twenty materials tested: one PVA rope and three nylon structures. The only other study that has examined a range of materials to determine their suitability as farming structures was done by Moore (1908a) who stated that explants of bath sponges will "speedily attach to any firm, clean, innocuous material". The unsuitability of natural fibre ropes to farm sponges because they rot in seawater and prevent explant attachment, supports his statement. However, the results of this study also indicate that the texture of the farming material can affect the attachment success of some sponge species. For example, explants of *L. brevis* attached to only 3 of the 7 structures of nylon composition (Table 4.12). All explants farmed using the other 4 structures either actively avoided contact through moving away from it or simply did not attach to the structure.

Several studies have discovered that the attachment rate of explants increases with increasing water temperature. Barthel and Theede (1986) recorded that the attachment of *Halichondria panicea* explants to glass slides took 14 days at 5°C but only 3 days at 15°C. Rosell and Uriz (1992) found that *Cliona viridis* explants attached to shell pieces in summer but not in winter. Although ambient water temperature may influence the attachment success of *L. brevis*, *P. croceus* and *R. agminata*, it cannot explain differences in attachment within experiments when all explants experienced similar water temperature.

Explant attachment is just one of many factors to be considered in developing a farming structure for metabolite production. Another consideration is variation in response between species to the farming structure, because a suitable structure for one species may not be suitable for another (Verdenal and Vacelet 1990, Duckworth et al. 1997, Pronzato et al. 1999).

Table 4.12. Attachment success of *L. brevis*, *P. croceus* and *R. agminata* to the farming materials tested in the six experiments. Codes: -, no attachment; +, <50% of explants attached; + +, >50% of explants attached; blank cell, not tested.

Farming materials	*L. brevis*	*P. croceus*	*R. agminata*
1. glass	-		
2. polyvinyl chloride (PVC)	-		
3. polypropylene pieces & rope	-		-
4. polyethylene	-		-
5. shell	-	-	-
6. pebbles	-		
7. slate	-	-	-
8. concrete-fibrelight composite	-		-
9. manila	-	-	-
10. sizel	-	-	-
11. nylon rope	-	-	-
12. cotton	-	-	-
13. hemp	-	-	-
14. polyvinyl alcohol (PVA)	+ +	+ +	+ +
15. nylon cable-tie	+	-	-
16. scallop lantern, nylon	-	-	-
17. pearl lantern, nylon	-	-	-
18. individual mesh bags-a, nylon	+ +	+	-
19. individual mesh bags-b, nylon	+ +	+	+
20. individual mesh bags-c, nylon	-	-	-

Considering all these requirements, and using the results from a series of experiments, two farming structures, rope and mesh arrays, were developed. The first structure involves placing explants on threaded PVA rope. This method is probably most suitable for hardy species which can survive the tissue damage incurred when rope is threaded through them. Because each explant is directly exposed to the environment, rope arrays will probably promote greatest growth. Mesh arrays farm explants in mesh stocking,

and probably are more suitable to use with soft, fleshy sponges which require a secure support on which to attach and grow. Attachment will promote survival but the surrounding mesh may restrict explant growth. A study is now required to determine the potential of rope and mesh arrays as farming structures for the commercial aquaculture of sponges for metabolite production.

Chapter 5. Farming *Latrunculia brevis* and *Polymastia croceus* commercially

5.1. Introduction

Several thousand tonnes of a sponge may be needed each year to supply sufficient quantities of a target bioactive metabolite for drug manufacture (Munro et al. 1999). Before sponge aquaculture is therefore accepted as a commercially viable method of supplying bioactive metabolites it must be demonstrated that adequate production of tissue and metabolite is possible.

The production of sponges, as for any cultured marine organism, will be determined by growth and survival, which are greatly affected by the farming environment. For example, Verdenal and Vacelet (1990) farmed three *Spongia* species at various distances away from a sewer outlet and discovered that explant growth and survival were both reduced in heavily-polluted water, probably because of a high sediment-load smothering the explants. Wilkinson and Vacelet (1979) transplanted six Mediterranean species to different conditions of exposure and discovered that explant growth generally increases as exposure increases. Duckworth et al. (1997) farmed *Psammocinia hawere*, *Raspailia agminata* and *Raspailia topsenti* at three depths in two exposures. They found that both explant growth and survival were generally poorest at the shallow depth and at the high exposure, probably due to the damaging effects of high light levels and strong water movement. This study provides more detail on how the environment can influence the farming of sponges. Explants of *Latrunculia brevis* and *Polymastia croceus* were transplanted and farmed for two months in each season at different exposures and depths (Chapter 3). Although *L. brevis* and *P. croceus* responded differently to transplanting, growth and survival were generally greatest in winter and spring when food is abundant and water temperature is low. Low water temperature in winter (9°C) probably reduces explant stress during transplanting. In addition, growth generally increased as exposure increased because of greater food availability. Exposure appears to be a key factor in many of these studies and long-term experiments examining its effects will be required to maximise the production from a commercial sponge farm.

The environment can also influence metabolite biosynthesis in sponges. Thompson et al. (1987) found that explants of *Rhopaloeides odorabile* produce more diterpenes

when grown in high light conditions, possibly to inhibit surface overgrowth of fouling organisms. Kreuter et al. (1992) also found that high light conditions can promote metabolite biosynthesis in *Aplysina* (*Verongia*) *aerophoba*. In this study, however, neither the farming season, exposure nor depth affected metabolite biosynthesis of *L. brevis* and *P. croceus* (Chapter 3). Bioactivity was generally greater in explants than in wild sponges, probably in response to tissue damage from cutting, which may have obviated any differences between the environmental conditions.

In aquaculture, production is greatly influenced by the farming structure used (Lutz 1980). The development of a suitable structure for farming sponges has been explored in several studies. Bath sponges farmed on threaded insulated wire or on concrete discs could double in volume (Moore 1908a) or increase by 150% (Crawshay 1939) over one year. Similar growth rates have been achieved in *Spongia* species threaded onto plastic-coated wire (Verdenal and Vacelet 1990). Only recently have studies explored farming structures for sponge metabolite production (Battershill and Page 1996, Duckworth et al. 1997, Munro et al. 1999). This study compared growth, survival and biosynthesis among explants of three species farmed inside mesh, attached to rope lengths, on threaded rope and with rope wrapped around them (Chapter 4). Two structures, rope and mesh arrays, were developed from this study but require further examination to determine their commercial potential for farming sponges.

Many sponge species have a good ability to survive and regrow lost tissue resulting from storm damage or predator attack (Ayling 1981, Hoppe 1988, Battershill and Bergquist 1990). Therefore, it may be possible to capitalise on this regenerative ability and harvest an explant many times, thereby increasing overall production of sponge tissue and bioactive metabolites. Harvesting would involve the cutting and removal of new tissue, leaving behind the original explant "core" to heal and regrow. Regular harvesting of the same farmed individual is possible for some algal species. For example, plants of *Eucheuma alvarezii* (Rhodophyta) can be harvested every few months to provide a regular and continuous supply of carrageenans for the food industry (Doty 1986). Harvesting involves the removal of the top portion of each thallus, and plants may survive continual harvesting for over 10 years. Because the farming season can influence the growth and survival of sponge explants (Moore 1908a, Battershill and Page 1986, Duckworth et al. 1997, Chapter 3) the time of harvesting can be important. In addition, metabolite

biosynthesis in wild sponges can vary with season (Green et al. 1990, Turon et al. 1996, Swearingen and Pawlik 1998) which may affect final metabolite production.

The density at which organisms are farmed is always an important consideration in aquaculture. As density increases beyond a critical point, growth decreases because food becomes limited (Holiday et al. 1991, Parsons and Dadswell 1992, Román et al. 1999). In sponges, the optimal farming density will be determined by overall production of tissue and metabolites, but, no study has explored this aspect of the optimal density for sponge culture.

In this chapter, the commercial potential of rope and mesh arrays for farming sponges for metabolite production is examined. This involved farming two species, *Latrunculia brevis* and *Polymastia croceus*, for nine months at two exposures, and harvesting them at different times. Harvesting involved the removal of new tissue leaving behind the sponge "core" to regrow. Sponge growth, survival and biosynthesis were monitored throughout the study. In a separate experiment, the effect of density on the growth and survival of *L. brevis* and *P. croceus* in rope and mesh arrays was examined. The commercial potential of rope and mesh arrays was further tested by farming *Mycale* sp. *Raspailia agminata* and *Polymastia massilis* in both array types.

5.2. Methods

5.2.1. Farming and harvesting *Latrunculia brevis* and *Polymastia croceus*

5.2.1.1. Collecting and cutting sponges

Sixty *L. brevis* and 60 *P. croceus* were collected, leaving one third of the individual behind, from wild populations on the south coast of Wellington in September `98. These sponges were cut using a sharp scalpel into cube-shaped explants, approximately 27cm^3 in volume, with one uncut side covered in intact pinacoderm and five cut sides initially showing exposed mesohyl.

Before farming, 50 explants of each species were wet-weighed and measured to determine their mean initial weight and volume. Except during weighing, all explants were kept in running seawater.

5.2.1.2. Farming layout and arrays

L. brevis and *P. croceus* were farmed at two exposures differing in their degree of water movement: high exposure (Barrett Reef) at the entrance to Wellington Harbour, and moderate exposure (Mahanga Bay) within the harbour (Fig. 1.2, 5.1). A previous experiment comparing the erosion of plaster-of-paris discs showed that water movement differs significantly between the two exposures (Fig. 3.13). In each exposure, explants were farmed at a depth of 12m in two sites, approximately 50m apart (Fig. 5.1). Each site was further subdivided into three areas, about 5m apart. Into each area, 3 rope and 3 mesh arrays (see Fig. 4.8), each carrying an identification tag, were tied to a rope backline at 50cm intervals (Fig. 5.2). The arrays were randomly ordered and had their positions mapped. Each rope and mesh array contained five explants of each species. These explants were placed at 10cm intervals and separated from explants of the other species by at least 30cm to prevent inter-specific competition and chemical interaction. The species position on an array was randomised. In total, 360 explants of each species were farmed in 36 rope and 36 mesh arrays. This experiment ran for 285 days, from 11 September `98 to 23 June `99.

5.2.1.3. Explant harvesting

Explants of *L. brevis* and *P. croceus* were harvested at intervals after farming started. Explants farmed in the first rope and mesh array in each area were harvested in December `98, March `99 and June `99 (3, 6 and 9 months after farming started). These were called "triple-harvest explants" (Fig. 5.1). Explants in the second rope and mesh array in each area were harvested in March `99 and June `99 ("double-harvest explants"). While explants in the third and final rope and mesh array in each area were harvested once in June `99 ("single-harvest explants"). Using the same harvesting order between arrays in an area eliminated any possible confusion on which array to harvest among the 72 transplanted. It was assumed that this would not affect explant growth or survival among treatments.

All explants harvested in Dec `98 and March `99 were cut underwater still attached to their arrays. New tissue growth was removed with a sharp scalpel, returning each explant to its original cube shape and volume. No explants smaller than $27cm^3$ were harvested. All tissue harvested from each species farmed in a single array was placed into a separate labelled bag and weighed on return to the laboratory. The tissue was then frozen

for future chemical analysis. In June '99, nine months after farming started, all arrays were removed and all explants were harvested and weighed.

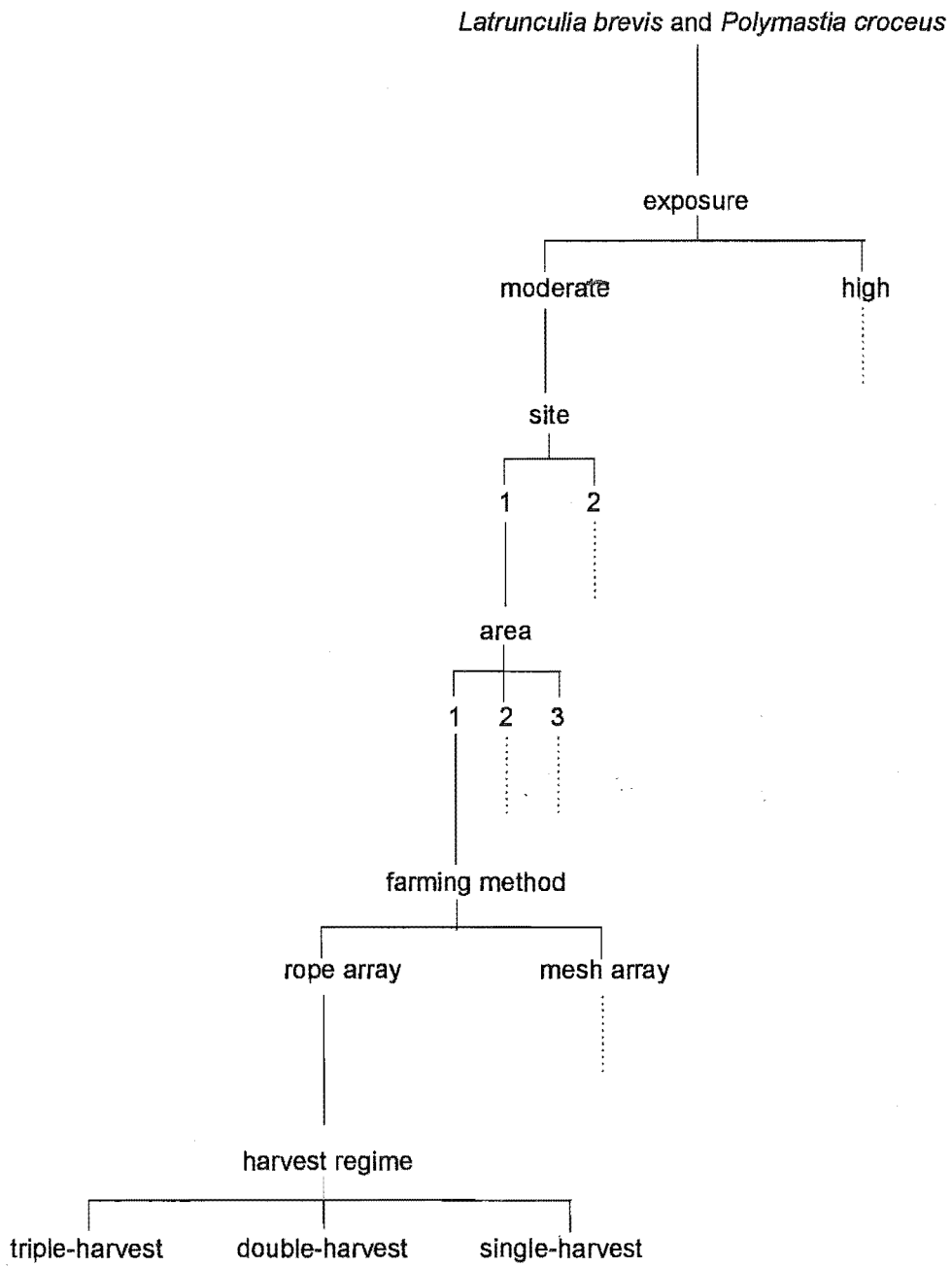


Figure 5.1. Experimental design for farming *L. brevis* and *P. croceus* between exposures (sites and areas nested), farming methods and harvest regimes. The design is orthogonal but for clarity only one full line is shown. Dotted lines indicate where the design is mirrored.

Figure 5.2. Photograph showing the rope and mesh arrays situated ~50cm apart. The orange explants are *P. croceus* and the green explants are *L. brevis*. The fish *Notolabrus celidotus* can be seen grazing on organisms fouling a mesh array. The photo was taken in February `99, 5 months after farming started. Black bar represents 10cm.

5.2.1.4. Monitoring growth and survival

Approximately every month explants were monitored *in situ,* with their survival and volume being recorded. Monitoring started from the bottom of each array, so each explant could be individually tracked and monitored over the experimental period. The length, width and height of each explant was measured with a ruler to the nearest half centimetre. Multiplying these measurements calculated explant volume. Explant shape, defined as "box" or "wedge", was also recorded. Most explants remained approximately cube-shaped, while others became more wedge-shaped, with a triangular profile. In the case of the latter, the calculated cubic voume was halved to give a better estimate of "wedge" volume.

One possible source of error in estimating the volume of explants was that both species could inflate in size, possibly when feeding. However, cleaning and removing fouling organisms, such as bushy bryozoans and hydroids, from each array immediately before monitoring caused inflated explants to shrink to their deflated volume, and eliminated this problem.

5.2.1.5. Monitoring bioactivity

At the end of the experiment, explants of *L. brevis* and *P. croceus* were analysed using a P388 murine leukaemia bioassay to determine whether the method of farming, harvest regime, or exposure influenced bioactivity. The sampling and analysis procedure is described in Chapter 2. The bioactivity of harvested tissue at each harvest date (Dec `98, Mar `99 and Jun `99) was also compared to determine whether repeated harvesting of *L. brevis* and *P. croceus* affected their bioactivity. Because of the expense of doing chemical analyses, explants from only one exposure (high) were used.

5.2.1.6. Statistical analysis

Analysis of variance was used to determine statistically whether the farming response of *L. brevis* and *P. croceus* differed significantly between treatments. Percentage survival, overall tissue yield per array and bioactivity were compared between exposures, sites (nested), farming methods and harvest regimes. The percentage survival was the number of explants surviving from the 5 transplanted per array. The overall tissue yield was the overall weight of tissue harvested per array. Final weights of single-harvest explants were compared between exposures, sites (nested) and farming methods. For final

weights, area was not tested because mortality was high in some farming treatments resulting in low replicate number; combining the final weights from the three areas at each site increased the power of the ANOVA model (Zar 1999). Although final weight was used to examine treatment effects statistically, growth of both species over the experimental period is graphically shown as explant volume (cm^3). Linear regression analysis determined that there is strong relationship between final explant weight and volume for both small and large *L. brevis* and *P. croceus* (Fig. 5.3). To examine the effect of harvesting on growth the mean monthly growth rate of the 3 harvest regimes after the December and March harvests were compared statistically. The mean monthly growth rate was determined by the formula:

$$\text{growth rate} = \left((\text{volume}_{3\text{months}} - \text{volume}_{\text{postharvest}}) / \text{volume}_{\text{postharvest}}\right) / 3$$

where $\text{volume}_{\text{postharvest}}$ and $\text{volume}_{3\text{months}}$ are the volume of an explant immediately after harvesting and 3 months later, respectively. This formula was adapted from Turon et al. (1998). After each ANOVA, all significant factors were further tested with the *a posteriori* Tukey-Kramer Multiple Comparison test to determine which treatments were significantly different.

5.2.1.7. Physical conditions

The water temperature at a depth of 5m in the site of moderate exposure was recorded daily by the NIWA Mahanga Bay Hatchery. Over the farming period, the water temperature increased from 12°C in September `98, to peak at 19°C in January, and then decreased to 13°C by June `99 (Fig. 5.4). The water temperature at the December `98 and March `99 harvests was 16°C and 18°C respectively. Previous monitoring had determined that the water temperature was similar between the two exposures (Fig. 3.12b).

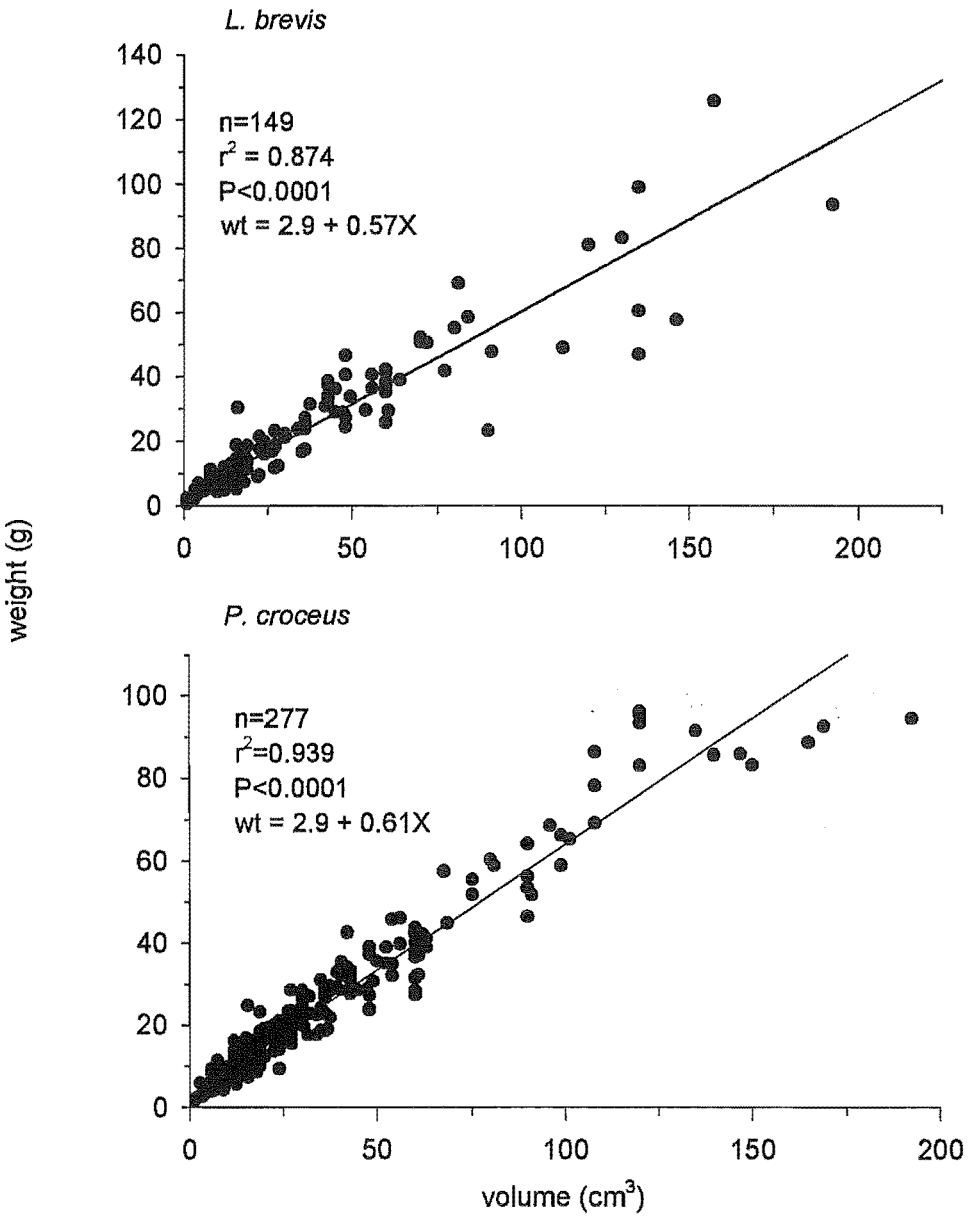


Figure 5.3. Linear regression between final explant volume and weight for *L. brevis* and *P. croceus*.

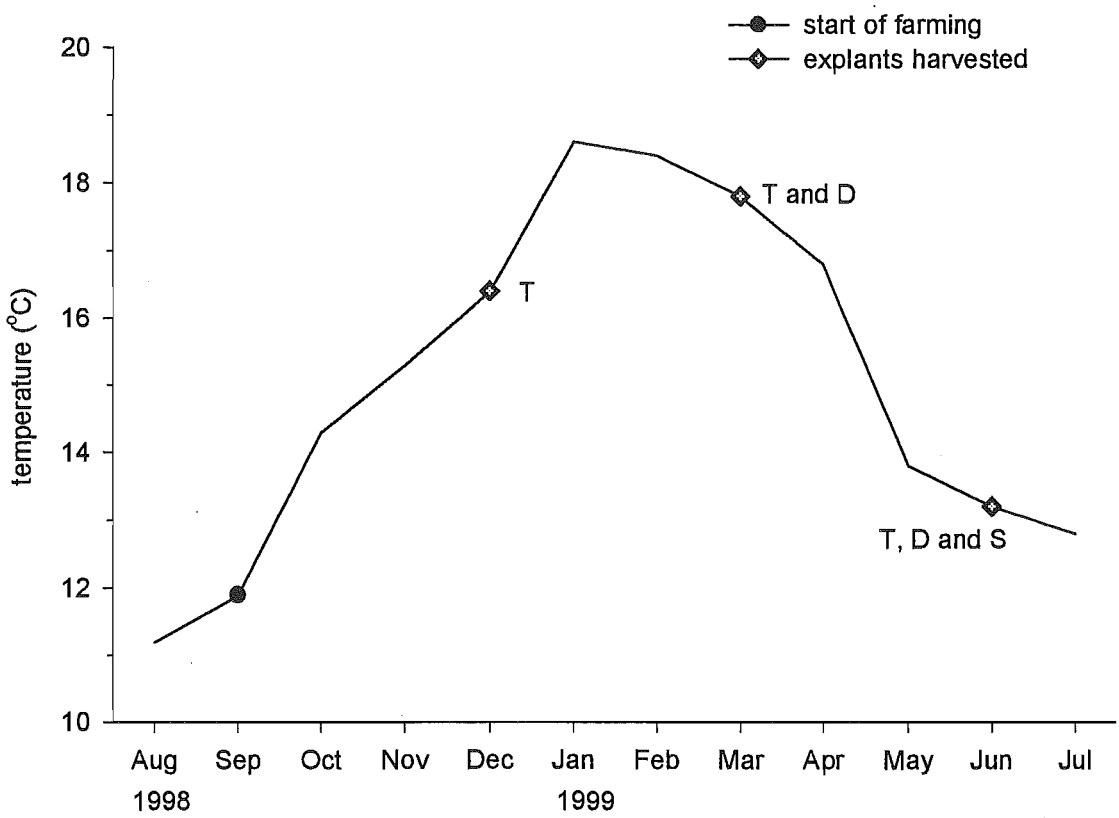


Fig. 5.4. Water temperature in Wellington Harbour from August '98 to July '99. Harvest regimes: T = triple-harvest explants, D = double-harvest explants, S = single-harvest explants.

5.2.2. Optimal farming density in the rope and mesh arrays

An additional 20 *L. brevis* and 20 *P. croceus* were collected from the south coast of Wellington in September `98 and cut into ~27cm^3 explants. These explants were farmed in rope and mesh arrays at three densities: "high density", with explants farmed at 5cm intervals; "low density", explants farmed at 20cm intervals; and, "medium density", explants farmed at 10cm intervals. Thus, explant separation halved as density increased. Each array-density combination had 5 explants of each species. Explants of the two species were separated by at least 30cm to prevent interspecific competition and chemical interaction. A species order or position on an array was randomised. Three rope and 3

mesh arrays of each farming density were tied to a rope backline at a depth of 12m at one site at the moderate exposure (Mahanga Bay). The site was divided into 3 areas, about 5m apart. One rope and mesh array of each density was placed into each area. This experiment ran for 285 days, from 11 September `98 to 23 June `99.

5.2.3. Examining the commercial potential of rope and mesh arrays for other sponge species

To further examine the commercial potential of the two farming methods the species *Mycale* sp., *Polymastia massilis* and *Raspailia agminata* were farmed in rope and mesh arrays. Five explants (~27cm^3) of each species were farmed at 10cm intervals in each method. One rope and one mesh array containing one species only was attached to a rope backline at a depth of 12m at the moderate exposure (Mahanga Bay). *P. massilis* was collected from the South Coast of Wellington. *Mycale* sp. and *R. agminata* were collected from Cape Rodney (Fig. 1.2). Both species are rare on the South Coast of Wellington. This experiment ran for 123 days, from 15 October `98 to 15 February `99.

5.3. Results

5.3.1. Farming and harvesting *Latrunculia brevis* and *Polymastia croceus*

5.3.1.1. *Latrunculia brevis*

The survival of *L. brevis* differed significantly between the two farming methods (Table 5.1). Final survival was greater in the mesh arrays (59%) than in the rope arrays (23%) (Fig. 5.5). Explant survival in the rope arrays decreased immediately after transplanting in September `98, while survival in the mesh arrays was good up to January `99 (Fig. 5.5).

One reason for the poor survival in the rope arrays was that many *L. brevis* explants rejected and moved away from the threaded PVA rope, eventually dislodging themselves. Only 10 of the 40 *L. brevis* explants that survived in the rope arrays to the end of the experiment attached to the threaded rope. These explants attached within 1 month. Almost half (13/30) of the explants that did not attach still grew, indicating that attachment is not required for growth. However, single-harvest explants that attached were heavier in final weight than explants that did not attach to the threaded PVA rope (One-Way ANOVA: $F_{df(1,7)}=9.14$, P=0.019). Another problem when farming *L. brevis* in the rope

arrays was that high water movement could tear large explants off the threaded rope. This was recorded for 14 large (>150cm^3) *L. brevis* explants farmed at the high exposure.

Table 5.1. Analysis of variance for survival of *L. brevis* between farming methods, exposures, sites (nested) and harvest regimes. GLM ANOVA used to analyse data. To meet assumptions, data were arcsine transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
method	1	4.054	4.054	47.18	0.021*
exposure	1	0.303	0.303	2.65	0.244
site (exposure)	2	0.228	0.114	0.97	0.385
method*exposure	1	0.038	0.038	0.4	0.574
method*site(exposure)	2	0.172	0.086	0.73	0.487
harvest	2	1.777	0.888	19.59	0.009*
method*harvest	2	0.402	0.201	5.5	0.071
exposure*harvest	2	0.704	0.352	7.76	0.042*
harvest*site(exposure)	4	0.181	0.045	0.39	0.818
method*exposure*harvest	2	0.36	0.18	4.93	0.083
method*harvest*site(exposure)	4	0.146	3.654	0.31	0.869
error	48	5.639	0.117		
total	71	14			

Unlike rope arrays, explant dislodgement in the mesh arrays was impossible because explants are effectively trapped inside the mesh. Many single-harvest explants grew partially through the mesh, incorporating the strands into their tissue. Tissue growth through the mesh started within 1 month after transplanting and allowed direct exposure of the explant to the outside environment (Fig. 5.6). For explants farmed in mesh, poor survival mostly resulted from tissue harvesting.

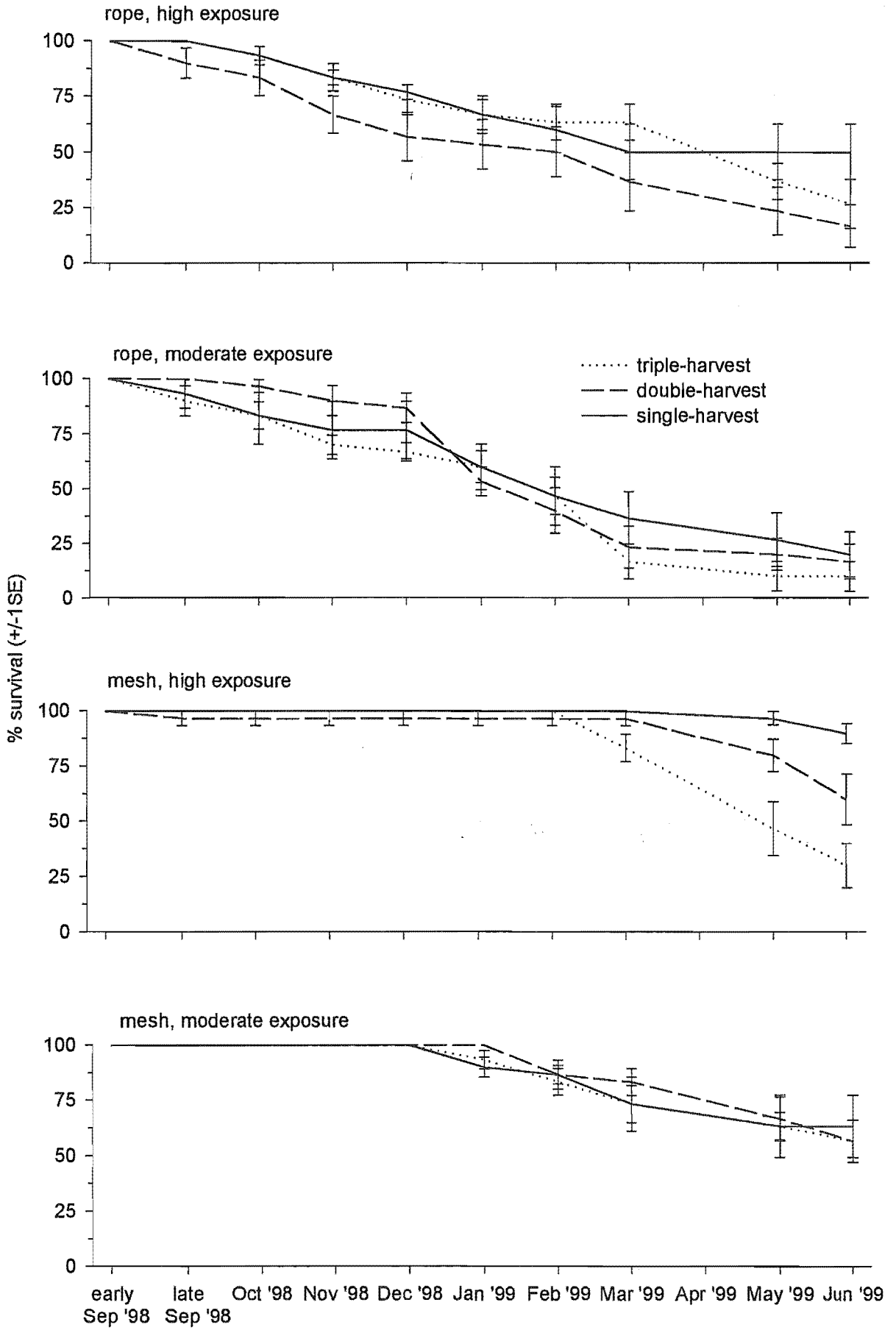


Figure 5.5. Percentage survival of *L. brevis* farmed in rope and mesh arrays at the high and moderate exposures from September '98 to June '99. Harvest regimes: triple-harvest harvested in Dec '98, Mar '99 and Jun '99; double-harvest in Mar '99 and Jun '99; and single-harvest in Jun '99 only. Error bars represent variation between arrays.

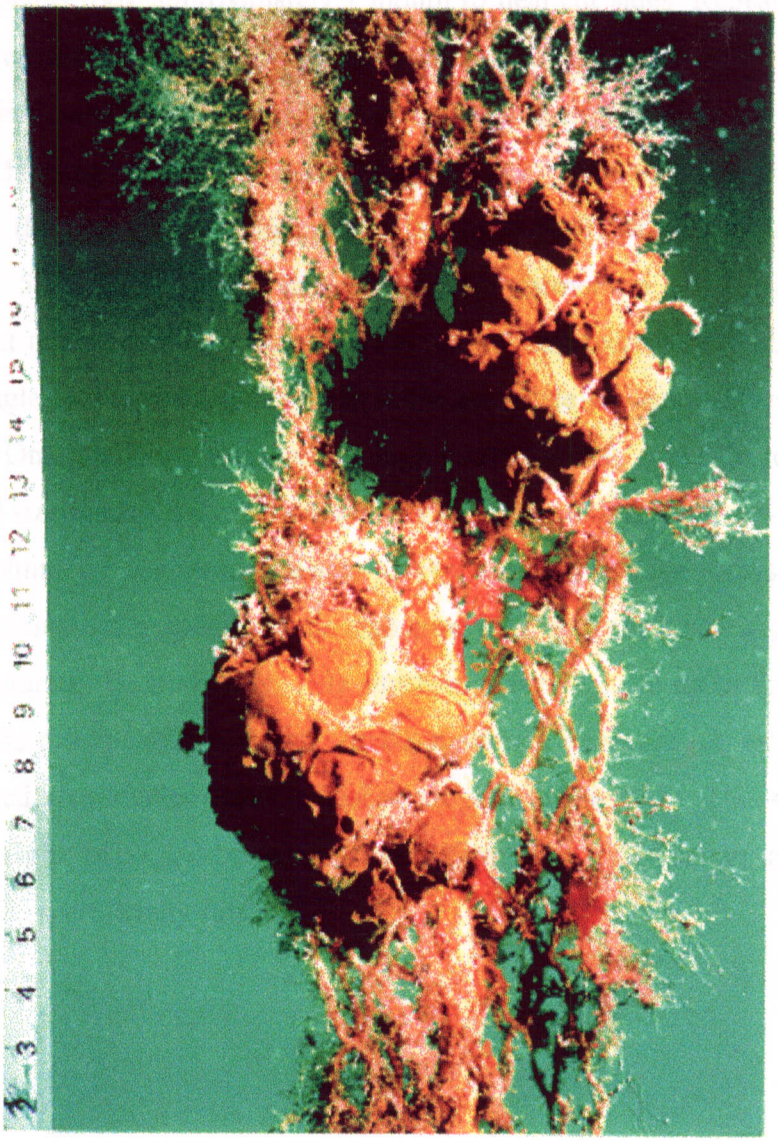


Figure 5.6. Photograph of *L. brevis* explants growing through the strands of a mesh array.The photo was taken in February `99, 5 months after farming started.

Survival after harvesting differed significantly between exposures (Table 5.1), being poorest at the high exposure (Fig. 5.5). Apart from this higher-order interaction, exposure did not affect the survival of *L. brevis* (Table 5.1). Overall, more single-harvest explants survived (56%) than double- (31%) and triple-harvest (38%) explants (Fig. 5.5). The effect of harvesting on survival differed over time because relatively more triple-harvest explants died after the March harvest than after the December harvest (Fig. 5.5). Harvested explants took approximately 1 month to heal cut sides. Repeated harvesting of triple-harvest explants did not influence this observed healing rate.

The final weights of single-harvest explants were affected significantly by the interaction of farming method and exposure (Table 5.2). Single-harvest explants farmed in rope arrays at the high exposure had grown by nearly 500% of their initial weight in nine months (Fig. 5.7). While single-harvest explants farmed in rope and mesh arrays, respectively, at the moderate and high exposures had approximately doubled their weight (Fig. 5.7). Single-harvest explants in mesh at the moderate exposure shrunk on average by 5g (Fig. 5.7). One possibility was that the high mortality of *L. brevis* in rope arrays could have distorted size means. To examine whether this occurred, the volume-frequency of the last known volume of disappearing single-harvest explants in rope arrays at the high and moderate exposure was graphed. At the high exposure, both small and large single-harvest explants died during the study (Fig. 5.8) which suggests that the mean final weight is not distorted by the disappearing explants. At the moderate exposure, all except one explant that disappeared was smaller than the final mean volume (Fig. 5.8). This suggests that the mean final weight of single-harvest explants farmed in rope arrays at the moderate exposure could be artificially small.

Table 5.2. Analysis of variance for final weights of single-harvest *L. brevis* between farming methods, exposures and sites (nested). GLM ANOVA used to analyse data. To meet assumptions, data were square root transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
method	1	63.99	63.99	254.2	0.0039*
exposure	1	87.61	87.61	34.26	0.0279*
site (exposure)	2	5.11	2.56	0.92	0.407
method*exposure	1	8.36	8.36	33.22	0.0288*
method*site(exposure)	2	0.503	0.252	0.09	0.914
error	46	128.29	2.789		
total	53	279.12			

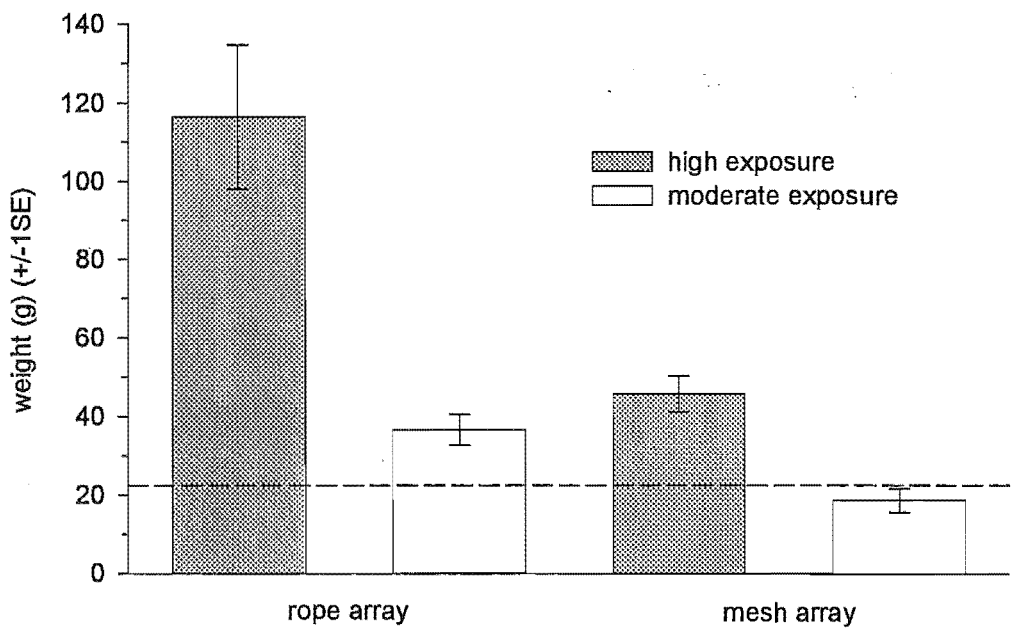


Figure 5.7. Mean final weight of single-harvest explants of *L. brevis* farmed in rope and mesh arrays at the high and moderate exposures. Dashed line represents the mean initial weight of explants. Error bars represent variation between explants.

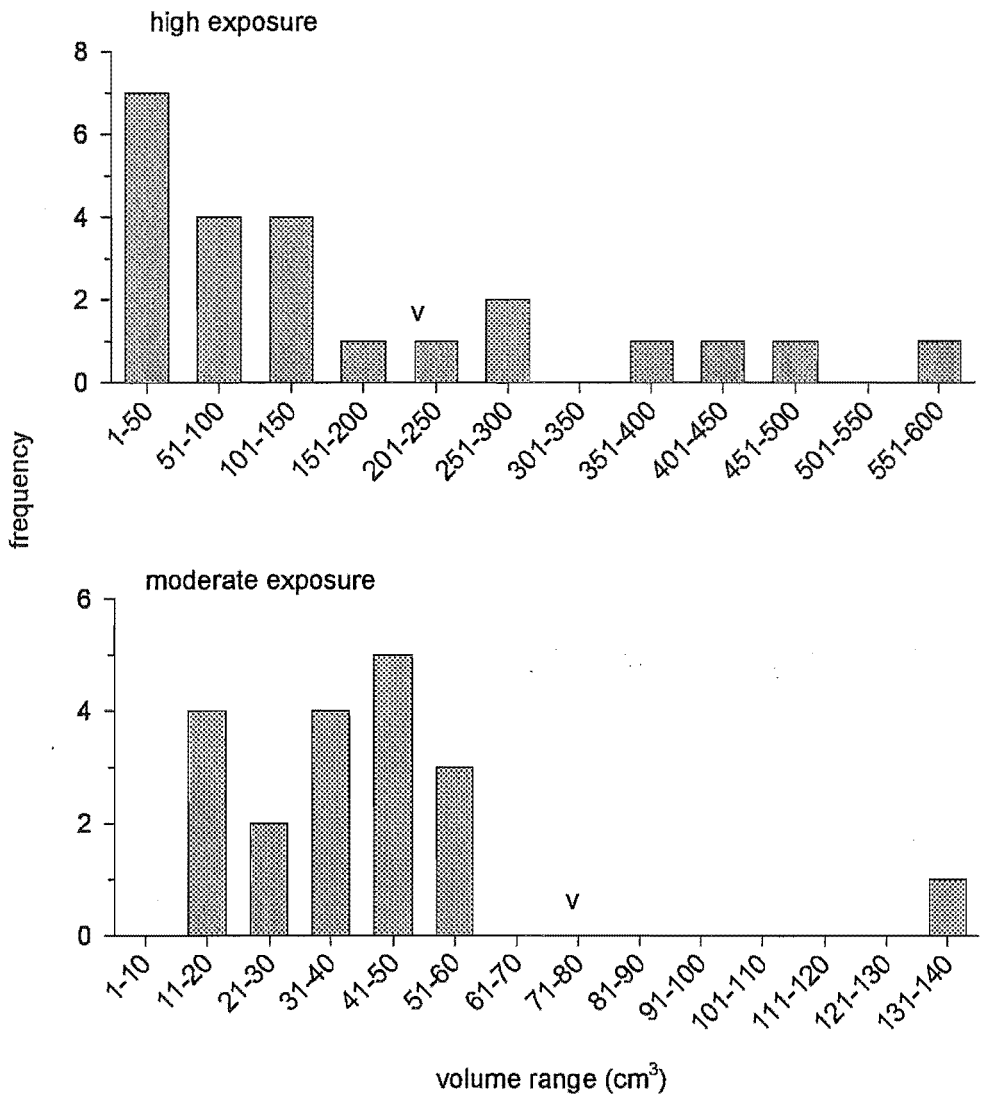


Figure 5.8. Volume frequency distribution of the last known volume of disappearing single-harvest explants of *L. brevis* farmed in rope arrays at the high and moderate exposures. The mean final volume (v) determined from Fig. 5.3 is shown for each exposure.

The results from Chapter 3 showed that explants farmed in similar conditions can vary greatly in final weight. Variation in final weight was examined in this study by plotting the weight frequency distribution of single-harvest *L. brevis* explants farmed in the mesh arrays at both exposures. (The final weight frequency distribution of single-harvest explants farmed in rope arrays is not shown because they had high mortality.) The results show that *L. brevis* explants farmed in very similar conditions can vary greatly in their final weight (Fig. 5.9). At both exposures, variation in final weight was similar between the two sites.

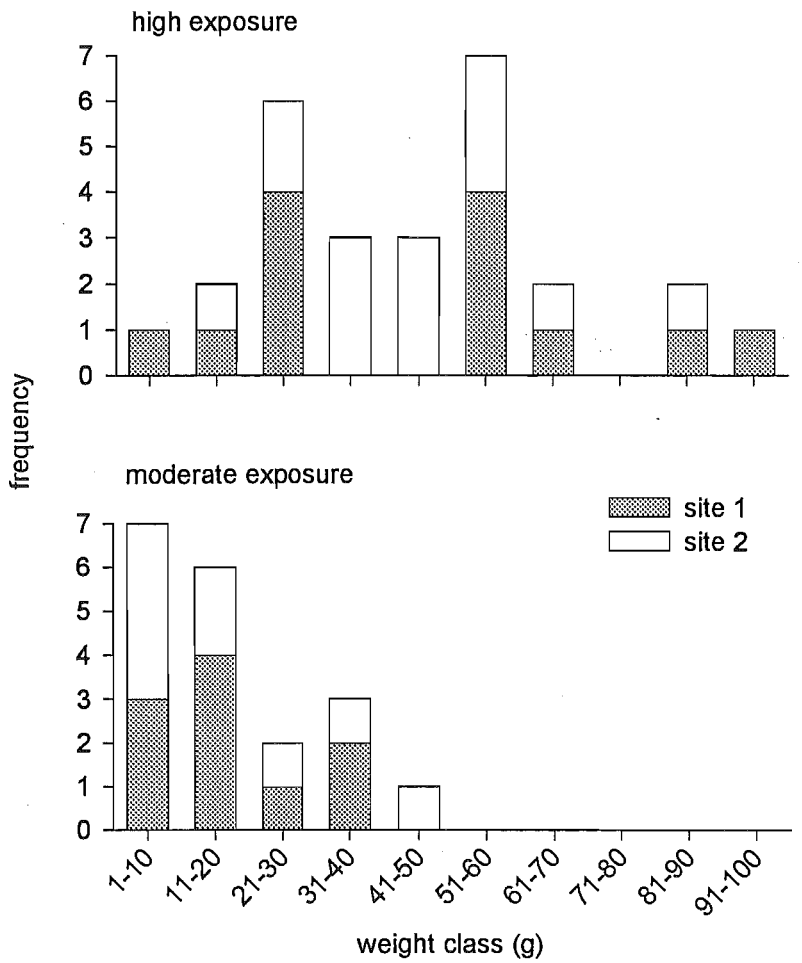


Figure 5.9. Final weight frequency distribution of single-harvest explants of *L. brevis* farmed in mesh at both exposures and separated into sites. Explants were 24g at the start of farming.

Growth of single-harvest explants varied over seasons. Generally, single-harvest explants grew from the start of spring (September `98) to reach a maximum volume by the end of summer (March `99) and then shrank over autumn (Fig. 5.10). This seasonal pattern of growth was most pronounced at the high exposure where overall growth was greatest. In March `99, single-harvest explants farmed in rope arrays at the high exposure had a mean volume of 241cm^3 (SE=40); the largest recorded explant was 567cm^3.

After the first harvest in December `98, triple-harvest explants grew at a similar rate to non-harvested explants (Table 5.3a) (Fig. 5.10). This indicates that harvesting *L. brevis* in early summer does not inhibit growth during a period when other sponges are growing. Growth rates differed significantly between methods (Table 5.3a), greatest in rope arrays (Fig. 5.10). Growth rates also differed between exposures (Table 5.3a), greatest at the high exposure (Fig. 5.10).

Table 5.3. Summary of the analysis of variances for growth rates of *L. brevis* after the December `98 and March `99 harvests. GLM ANOVA used to analyse data. To meet assumptions, both December `98 and March `99 post-harvest data were log transformed. Prob: * = significant.

	(a) Dec `98 post-harvest growth rates			(b) Mar `99 post-harvest growth rates		
Factor	DF	F-ratio	Prob	DF	F-ratio	Prob
method	1,2	19.28	0.048*	1,2	54.24	0.018*
exposure	1,2	95.88	0.01*	1,2	8.43	0.101
site (exposure)	2,190	1.66	0.194	2,108	0.77	0.463
method*exposure	1,2	7.57	0.111	1,2	0.00	0.998
method*site(exposure)	2,190	1.59	0.207	2,108	0.29	0.747
harvest	2,4	1.57	0.314	2,4	0.57	0.608
method*harvest	2,4	0.02	0.978	2,4	0.21	0.822
exposure*harvest	2,4	1.67	0.297	2,4	1.16	0.401
harvest*site(exposure)	4,190	0.31	0.873	4,108	2.04	0.093
method*exposure*harvest	2,4	0.4	0.692	2,4	0.29	0.761
method*harvest*site(exp.)	4,190	2.14	0.078	4,108	1.14	0.344

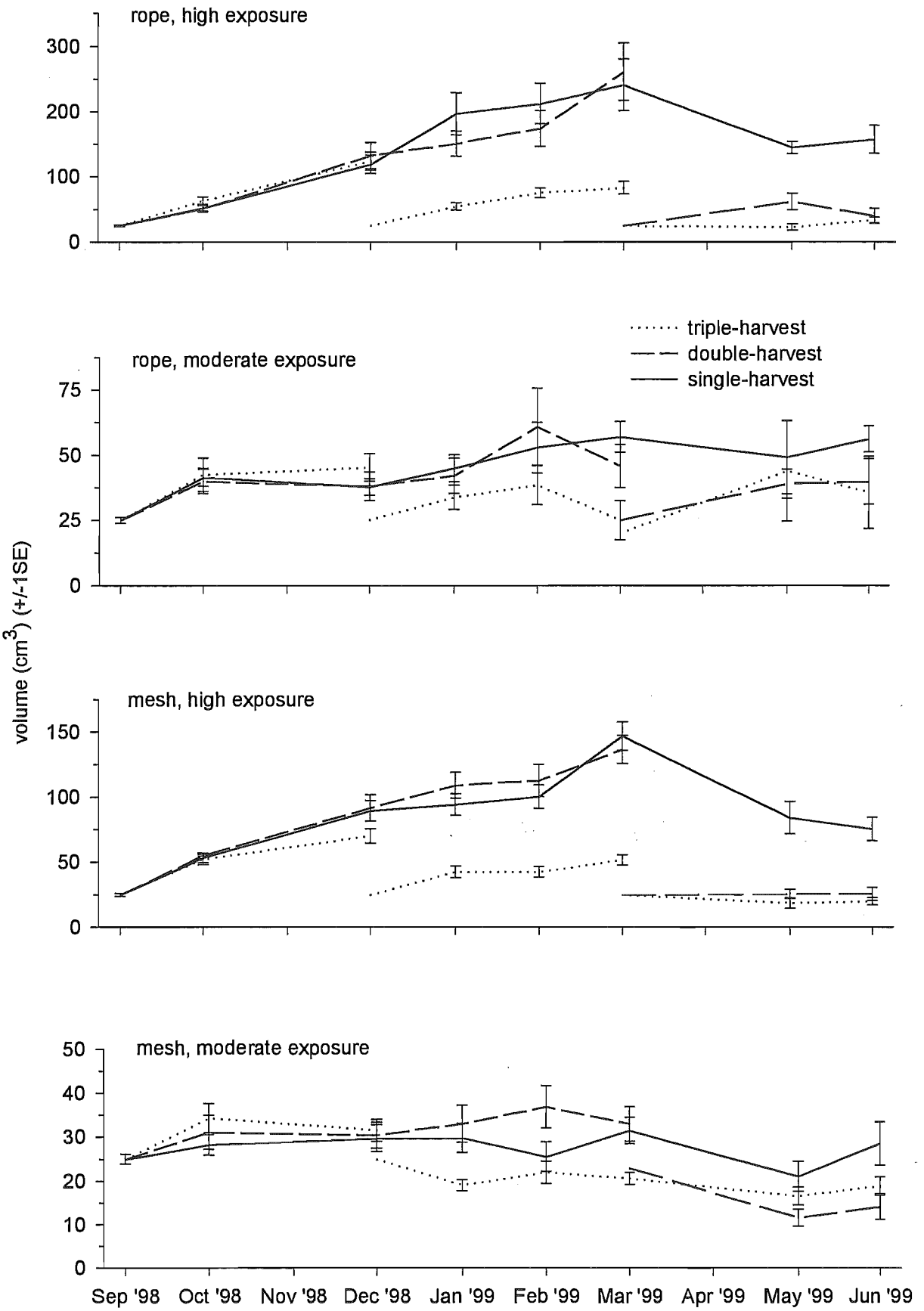


Figure 5.10. Mean volume of *L. brevis* farmed in rope and mesh arrays at the high and moderate exposures from September '98 to June '99. Harvest regimes: triple-harvest harvested in Dec '98, Mar '99 and Jun '99; double-harvest in Mar '99 and Jun '99; and single-harvest in Jun '99 only. Error bars represent variation between explants.

After harvesting in March `99, all harvest regimes had similar growth rates (Table 5.3b). However, the post-harvest growth rate differed significantly between methods (Table 5.3b), being greatest in the rope arrays (Fig. 5.10).

The overall tissue yield per array of *L. brevis* differed significantly between exposures (Table 5.4). The tissue yield from arrays situated at the high exposure was nearly three fold greater than from arrays at the moderate exposure (Fig. 5.11). There was a net loss of approximately half of initial transplanted weight from arrays situated at the moderate exposure.

Table 5.4. Analysis of variance for overall tissue yield per array of *L. brevis* between farming methods, exposures, sites (nested) and harvest regimes. GLM ANOVA used to analyse data. To meet assumptions, data were square root transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
method	1	139.58	139.58	21.18	0.044*
exposure	1	272.14	272.14	160.6	0.006*
site (exposure)	2	3.39	1.69	0.11	0.894
method*exposure	1	22.53	22.53	3.42	0.206
method*site(exposure)	2	13.17	6.59	0.44	0.648
harvest	2	21.63	10.82	2.49	0.198
method*harvest	2	112.46	56.23	10.9	0.024*
exposure*harvest	2	1.29	0.65	0.15	0.866
harvest*site(exposure)	4	17.38	4.34	0.29	0.844
method*exposure*harvest	2	64.42	32.21	6.24	0.059
method*harvest*site(exposure)	4	20.63	5.16	0.34	0.848
error	48	723.23	15.07		
total	71	1411.86			

Tissue yield was significantly affected by the interaction of farming method and harvest regime (Table 5.4). Single-harvest explants had greater yield when farmed in mesh arrays than in rope arrays (Fig. 5.11). Overall, tissue yield was greater in mesh arrays than in rope arrays (Fig. 5.11). Final weight, however, was greatest overall in the rope arrays

(Fig. 5.7). This demonstrates clearly that a farming method must promote both sponge growth and survival. Single-harvest explants farmed in mesh arrays at the high exposure had an average net gain of 80g per array (Fig. 5.11). This good tissue yield results from ≤5 explants and would have been substantially greater if the explants were totally harvested at their maximum volume in March '99.

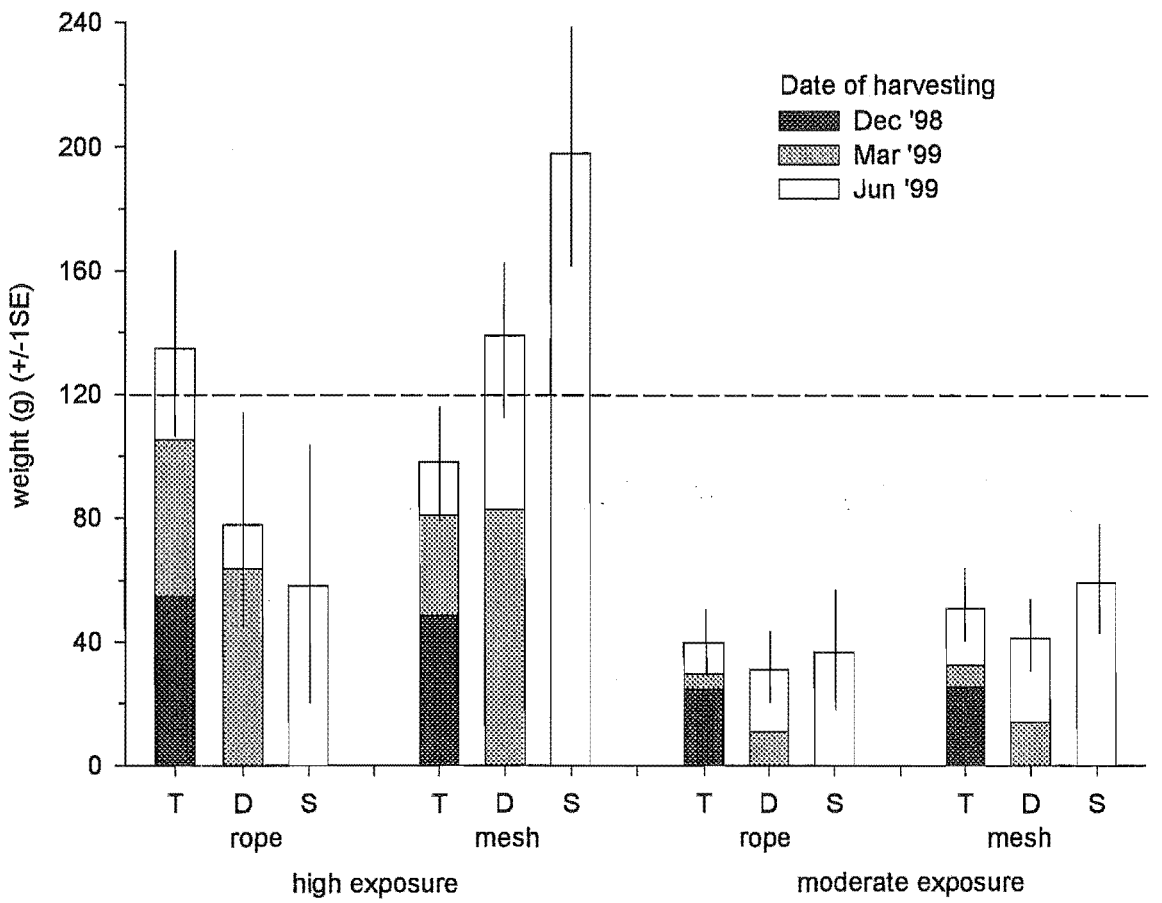


Figure 5.11. Mean tissue yield (g) per array (pooled across sites and areas) of *L. brevis* farmed in different exposures, farming methods, and harvest regimes: T=triple-harvest, D=double-harvest, and S=single-harvest. Dashed line represents the total initial weight of explants per array. Error bars represent the pooled variation between arrays.

Final explant bioactivity was similar between farming methods, exposures and harvest regimes (Table 5.5) (Fig. 5.12). Similar IC_{50} ranges between harvest dates within each harvest regime (Fig. 5.12) supports the statistical analysis that repeat harvesting did not affect final bioactivity. All samples, irrespective of treatment, were very active (<250ng/ml); an IC_{50} <1500ng/ml is considered very active (Lill et al. 1995).

Table 5.5. Summary of a series of One-Way ANOVA's examining bioactivity of *L. brevis* between farming methods, exposures and harvest regimes. Final bioactivity scores in June '99 were used to compare within each factor. Prob: * = significant.

Factor	DF	F-ratio	Prob
method	1,10	1.0	0.341
exposure	1,6	1.46	0.273
harvest	2,9	0.54	0.601

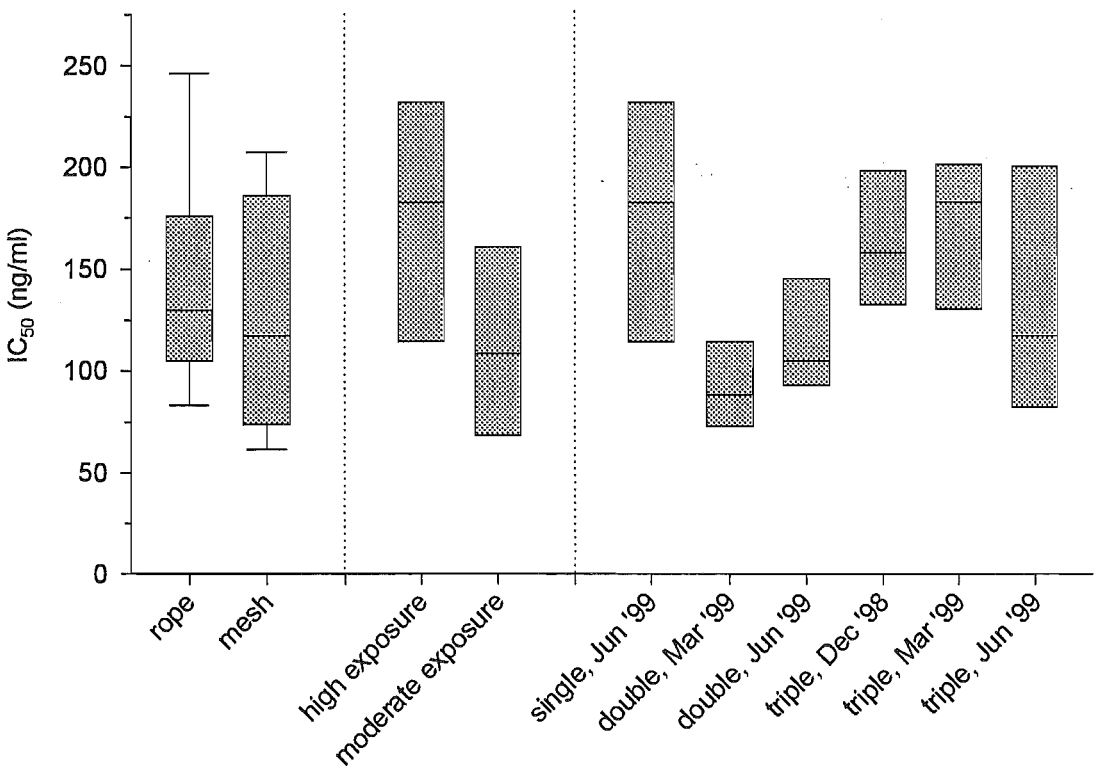


Figure 5.12. Final bioactivity (IC_{50}) of *L. brevis* between farming methods, exposures and harvest regimes. Harvest regimes further separated into harvest dates. Farming methods show the median, 5th and 95th percentiles (n=6 for each method). Both exposure and harvest regime show the range and median (n=4 for each treatment).

5.3.1.2. *Polymastia croceus*

The survival of *P. croceus* was influenced significantly only by the method of farming (Table 5.6). Final survival was greater for explants farmed in the mesh arrays (96%) than it was in the rope arrays (59%) (Fig. 5.13). Mortality was higher in rope arrays because some explants rejected the threaded PVA rope and subsequently dislodged themselves. Explant loss due to rope rejection was first recorded in December '98, three months after transplanting (Fig. 5.13). Therefore, good survival up to December '98 indicated that *P. croceus* can survive the tissue damage incurred when rope is threaded through them. By the end of the experiment, 69% of surviving explants had attached to the threaded PVA rope.

Table 5.6. Analysis of variance for survival of *P. croceus* between farming methods, exposures, sites (nested) and harvest regimes. GLM ANOVA used to analyse data. To meet assumptions, data were arcsine transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
method	1	9.945	9.945	56.27	0.017*
exposure	1	0.027	0.027	0.37	0.603
site (exposure)	2	0.146	0.073	0.5	0.607
method*exposure	1	0.089	0.089	0.51	0.551
method*site(exposure)	2	0.353	0.177	1.22	0.304
harvest	2	0.219	0.109	1.03	0.435
method*harvest	2	0.129	0.065	1.02	0.437
exposure*harvest	2	0.258	0.129	1.22	0.386
harvest*site(exposure)	4	0.423	0.106	0.73	0.574
method*exposure*harvest	2	0.346	0.173	2.74	0.178
method*harvest*site(exposure)	4	0.252	0.063	0.44	0.782
error	48	6.942	0.145		
total	71	19.13			

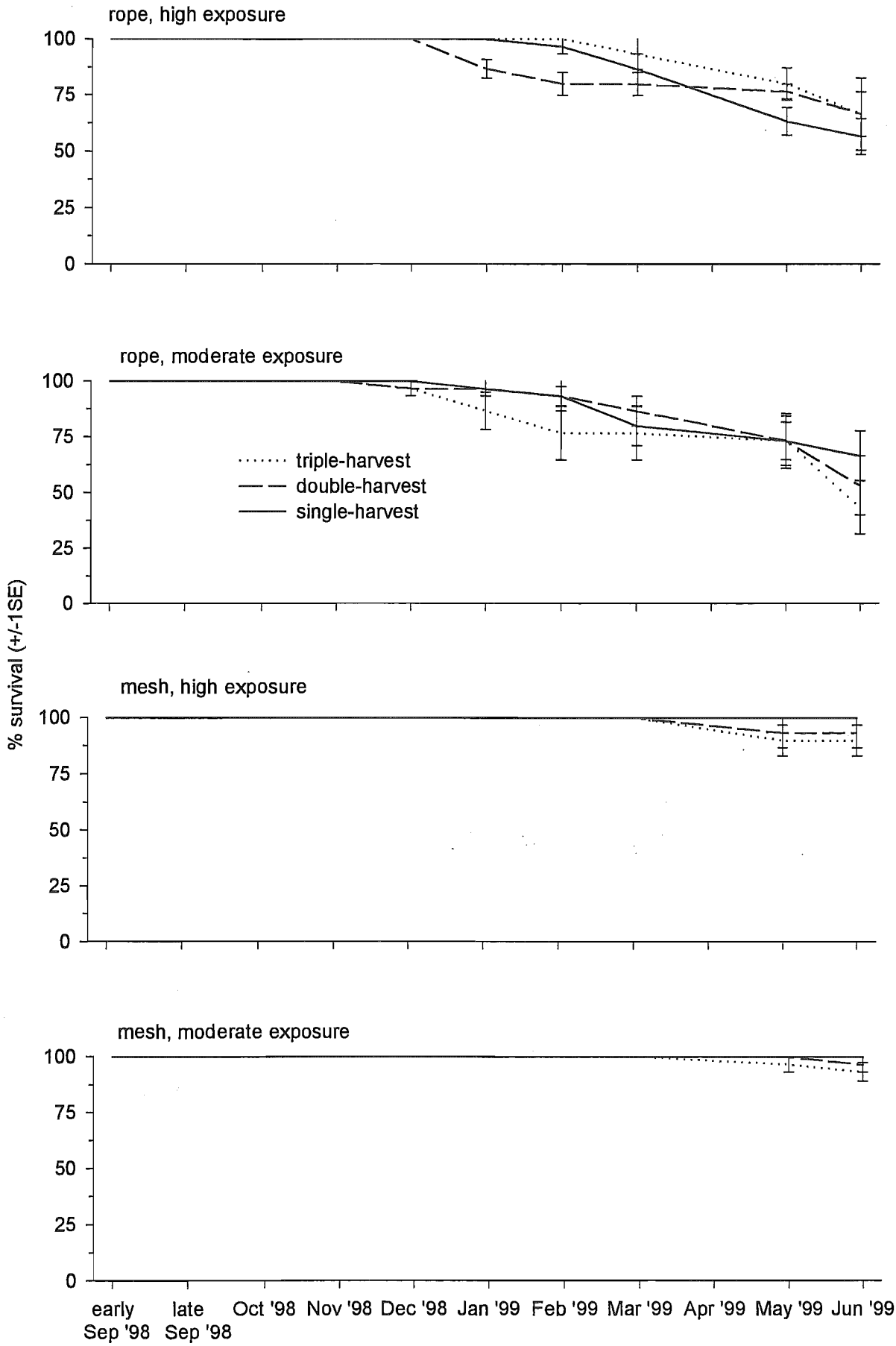


Figure 5.13. Percentage survival of *P. croceus* farmed in rope and mesh arrays at the high and moderate exposures from September '98 to June '99. Harvest regimes: triple-harvest harvested in Dec '98, Mar '99 and Jun '99; double-harvest in Mar '99 and Jun '99; and single-harvest in Jun '99 only. Error bars represent variation between explants.

Single-harvest explants that attached were heavier in final weight than explants that did not attach to the threaded rope (One-Way ANOVA: $F_{df(1,34)}=5.43$, P=0.026). Although many single-harvest explants farmed in the mesh arrays had grown to a large size, such that they filled out the mesh pocket they were contained in, only 30% grew through the mesh strands. For *P. croceus*, this involved the fusion of two neighbouring papillae around a mesh strand. This was very different from *L. brevis* where the whole explant grew through the mesh. This represents an important difference between the farming responses of *L. brevis* and *P. croceus*.

The harvested explants took up to 3 months to heal fully and look similar in appearance to the non-harvested explants. Repeated harvesting of triple-harvest explants had no noticeable effect on their healing rate.

The final weights of single-harvest explants differed significantly between farming methods (Table 5.7). The mean final weight of single-harvest explants was approximately twice as great in the rope arrays than it was in the mesh arrays (Fig. 5.14). Unlike the farming method, the final weights of single-harvest explants were similar between the two exposures (Table 5.7) (Fig. 5.14).

Table 5.7. Analysis of variance for final weights of single-harvest *P. croceus* between farming methods, exposures and sites (nested). GLM ANOVA used to analyse data. To meet assumptions, data were log transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
method	1	0.971	0.971	22.6	0.042*
exposure	1	0.033	0.033	0.87	0.448
site (exposure)	2	0.076	0.038	0.67	0.516
method*exposure	1	0.107	0.107	2.49	0.255
method*site(exposure)	2	0.086	0.043	0.76	0.471
error	89	5.039	0.056		
total	96	6.253			

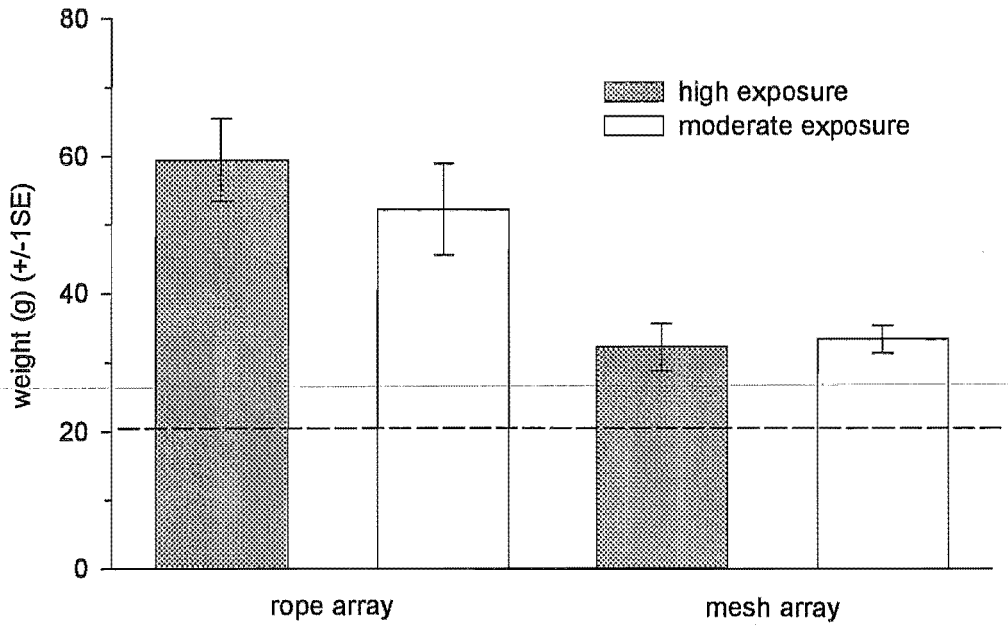


Figure 5.14. Mean final weight of single-harvest explants of *P. croceus* farmed in rope and mesh arrays at the high and moderate exposures. Dashed line represents the mean initial weight of explants. Error bars represent variation between explants.

The variation in final sizes of *P. croceus* was examined in this study by plotting the weight frequencies of single-harvest explants farmed in all treatments. The results show that *P. croceus* explants farmed in very similar conditions can vary greatly in their final weights (Fig. 5.15). Within each treatment, the variation in final weight was similar between the two sites.

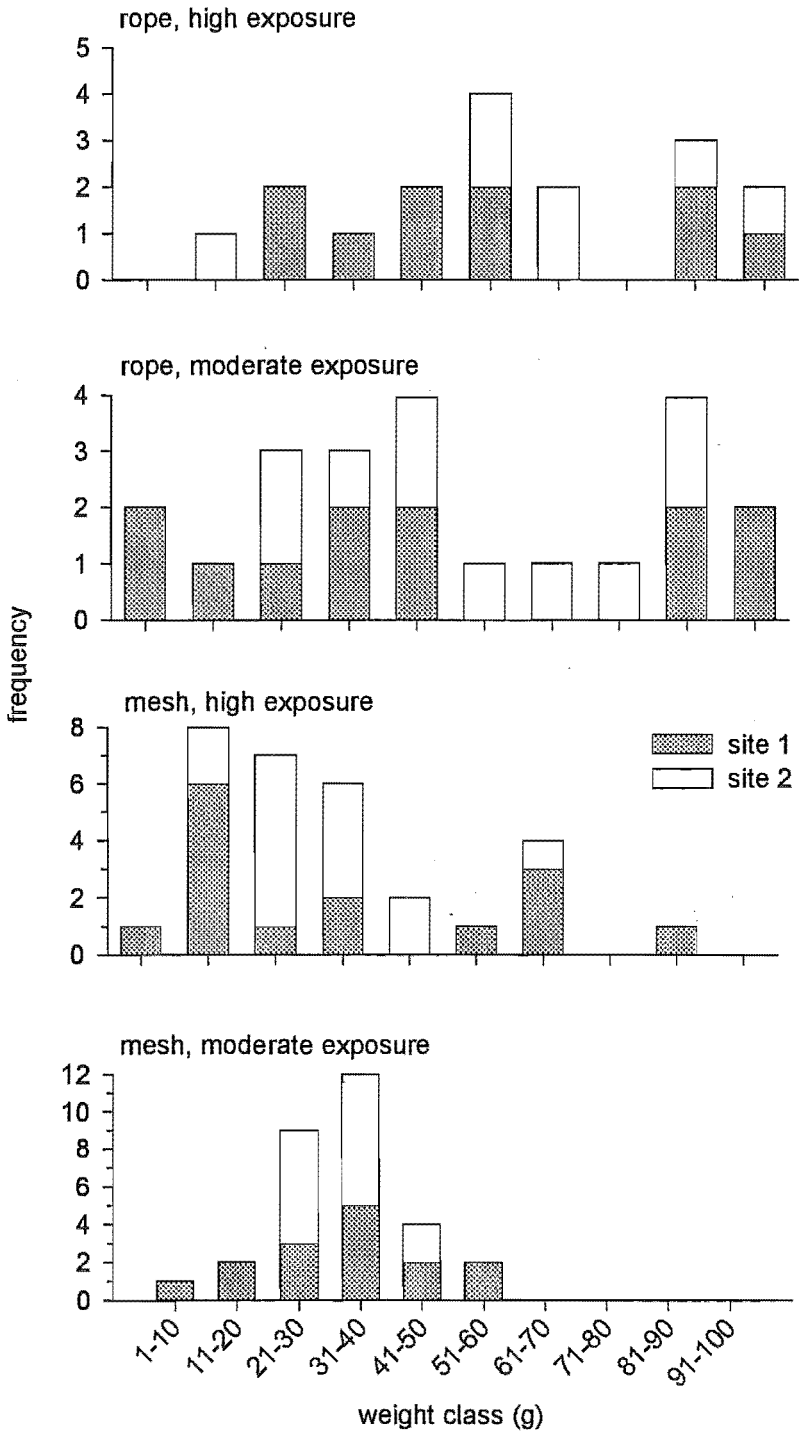


Figure 5.15. Final weight frequency distribution of single-harvest explants of *P. croceus* farmed in both methods and exposures, and separated into sites. Explants were 20g at the start of farming.

Growth of single-harvest explants varied between seasons. Generally, explants grew during spring to reach a maximum size in March `99 and then shrank over autumn (Fig. 5.16). In March `99, the volume of single-harvest explants differed significantly between the two exposures (Table 5.8), being greatest at the high exposure (Fig. 5.16). This indicates that growth of farmed *P. croceus* is influenced by the interaction of exposure and season. Explant volume in March `99 also varied between the farming methods (Table 5.8), greatest in rope arrays (Fig. 5.16). In March `99, the mean volume of single-harvest explants farmed in rope arrays at the high exposure was 170cm^3 (SE=18); the largest recorded explant was 409cm^3.

Table 5.8. Analysis of variance for volume of single-harvest *P. croceus* in March `99 between farming methods, exposures and sites (nested). GLM ANOVA used to analyse data. To meet assumptions, data were log transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
method	1	2.085	2.085	34.14	0.028*
exposure	1	2.108	2.108	22.71	0.041*
site (exposure)	2	0.187	0.0928	1.42	0.247
method*exposure	1	0.0453	0.0453	0.74	0.48
method*site(exposure)	2	0.122	0.0611	0.93	0.397
error	102	6.681	0.0655		
total	109	11.286			

After the first harvest in December `98, triple-harvest explants of *P. croceus* grew quickly (Fig. 5.16), but at a similar rate to non-harvested explants (Table 5.9a). This indicates that harvesting *P. croceus* in early summer does not inhibit growth during a period when other sponges are growing.

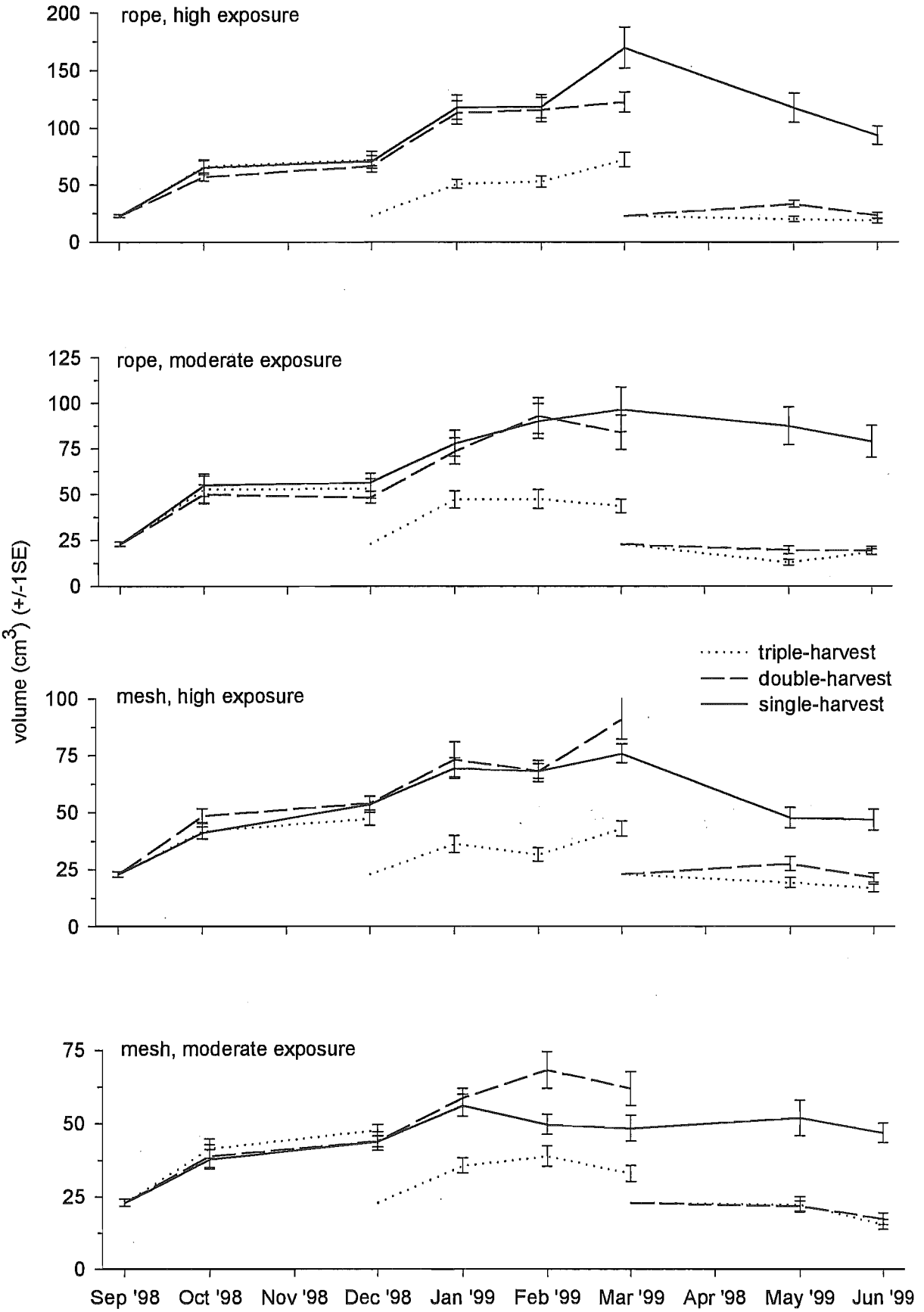


Figure 5.16. Mean volume of *P. croceus* farmed in rope and mesh arrays at the high and moderate exposures from September '98 to June '99. Harvest regimes: triple-harvest harvested in Dec '98, Mar '99 and Jun '99; double-harvest in Mar '99 and Jun '99; and single-harvest in Jun '99 only. Error bars represent variation between explants.

After the March `99 harvest, the mean growth rates of the three harvest regimes differed at the high exposure, resulting in significant exposure*harvest interaction (Table 5.9b). By June `99, single-harvest explants at the high exposure had shrunk by ~40%, while harvested explants were similar in volume to when they were cut in March `99 (Fig. 5.16)

Table 5.9. Summary of the analysis of variances for growth rates of *P. croceus* after the December `98 and March `99 harvests. GLM ANOVA used to analyse data. To meet assumptions, both December `98 and March `99 post-harvest data were log transformed. Prob: * = significant.

	(a) Dec `98 post-harvest growth rates			(b) Mar `99 post-harvest growth rates		
Factor	DF	F-ratio	Prob	DF	F-ratio	Prob
method	1,2	34.15	0.028*	1,2	0.46	0.569
exposure	1,2	56	0.017*	1,2	3.37	0.208
site (exposure)	2,307	066	0.518	2,253	1.39	0.252
method*exposure	1,2	1.05	0.414	1,2	0.01	0.915
method*site(exposure)	2,307	1.06	0.348	2,253	2.13	0.121
harvest	2,4	2.65	0.185	2,4	2.55	0.193
method*harvest	2,4	1.26	0.377	2,4	0.23	0.806
exposure*harvest	2,4	0.51	0.633	2,4	7.17	0.048*
harvest*site(exposure)	4,307	0.83	0.506	4,253	0.75	0.557
method*exposure*harvest	2,4	0.92	0.469	2,4	1.45	0.336
method*harvest*site(exp.)	4,307	0.88	0.476	4,253	1.58	0.179

The overall tissue yield of *P. croceus* was similar between farming methods, exposures and harvest regimes (Table 5.10). In all treatments, tissue yield surpassed initial transplanted weight (Fig. 5.17). Over half (7/12) of the treatments had an average net gain >50g per array. This represents a 50% increase in sponge weight over 9 months.

Table 5.10. Analysis of variance for overall tissue yield of *P. croceus* between farming methods, exposures, sites (nested) and harvest regimes. GLM ANOVA used to analyse data. To meet assumptions, data were square root transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
method	1	5.735	5.735	0.54	0.534
exposure	1	2.766	2.766	0.61	0.516
site (exposure)	2	9.032	4.516	0.76	0.472
method*exposure	1	4.259	4.259	0.4	0.592
method*site(exposure)	2	21.361	10.68	1.8	0.176
harvest	2	16.529	8.264	5.79	0.066
method*harvest	2	3.016	1.508	0.68	0.557
exposure*harvest	2	4.751	2.375	1.67	0.298
harvest*site(exposure)	4	5.707	1.427	0.24	0.914
method*exposure*harvest	2	13.181	6.591	2.97	0.162
method*harvest*site(exposure)	4	8.883	2.221	0.37	0.825
error	48	284.402	5.925		
total	71	379.622			

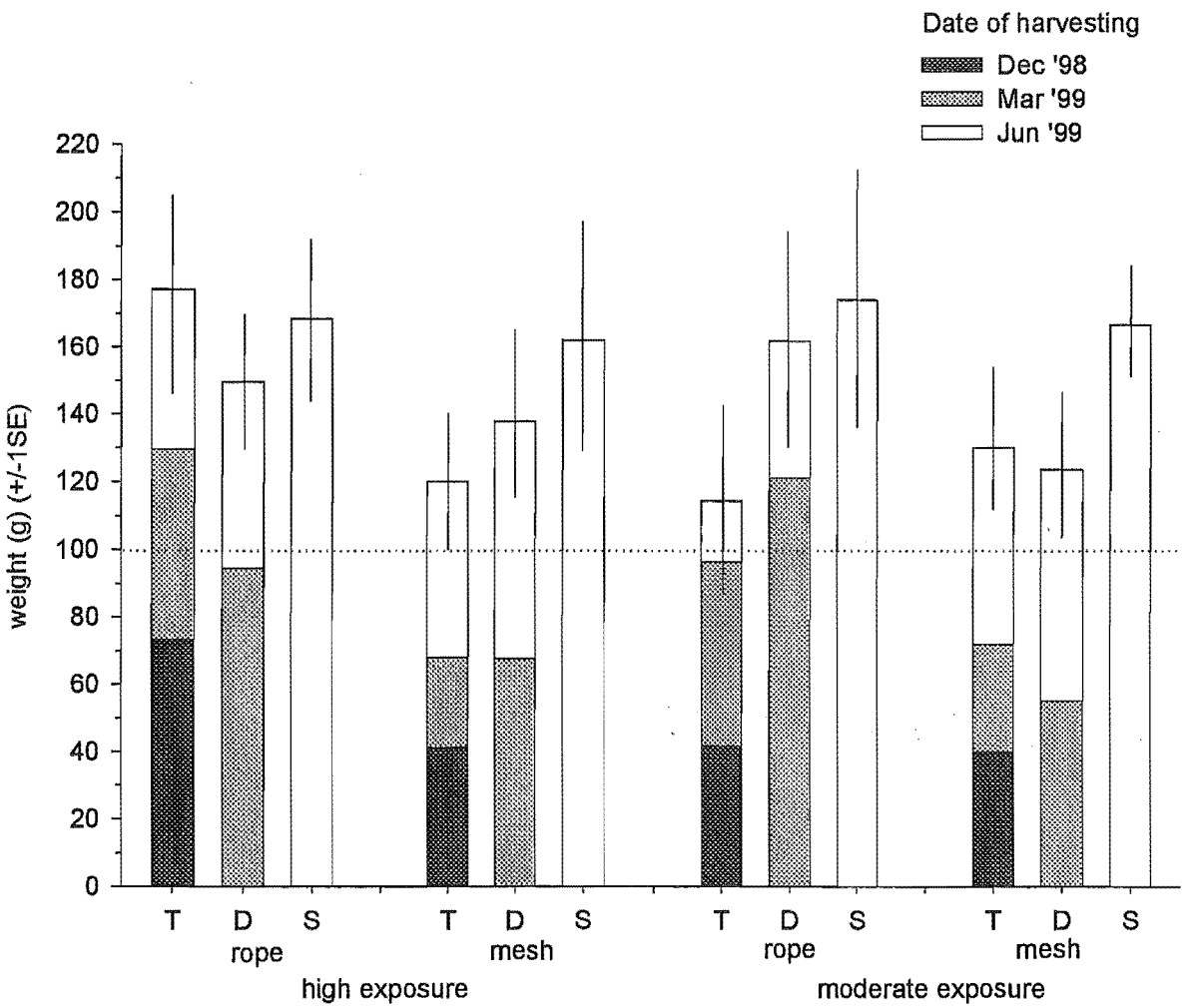


Figure 5.17. Mean tissue yield (g) per array (pooled across sites and areas) of *P. croceus* farmed in different exposures, farming methods, and harvest regimes: T=triple-harvest, D=double-harvest and S=single-harvest. Dashed line represents the total initial weight of explants per array. Error bars represent pooled variation between arrays.

Final explant bioactivity was similar statistically between farming methods, exposures and harvest regimes (Table 5.11) (Fig. 5.18). Within each harvest regime, the median IC_{50} at each date tended to be in the range of 80-200ng/ml (Fig. 5.18). This suggests that repeated harvesting did not promote bioactivity. As in the case of *L. brevis*, all samples, irrespective of treatments, were very active (<800ng/ml).

Table 5.11. Summary of a series of One-Way ANOVA's examining bioactivity of *P. croceus* between farming methods, exposures and harvest regimes. Final bioactivity scores in June `99 were used to compare within each factor. Prob: * = significant.

Factor	DF	F-ratio	Prob
method	1,10	0.97	0.349
exposure	1,6	4.85	0.069
harvest	2,9	2.65	0.124

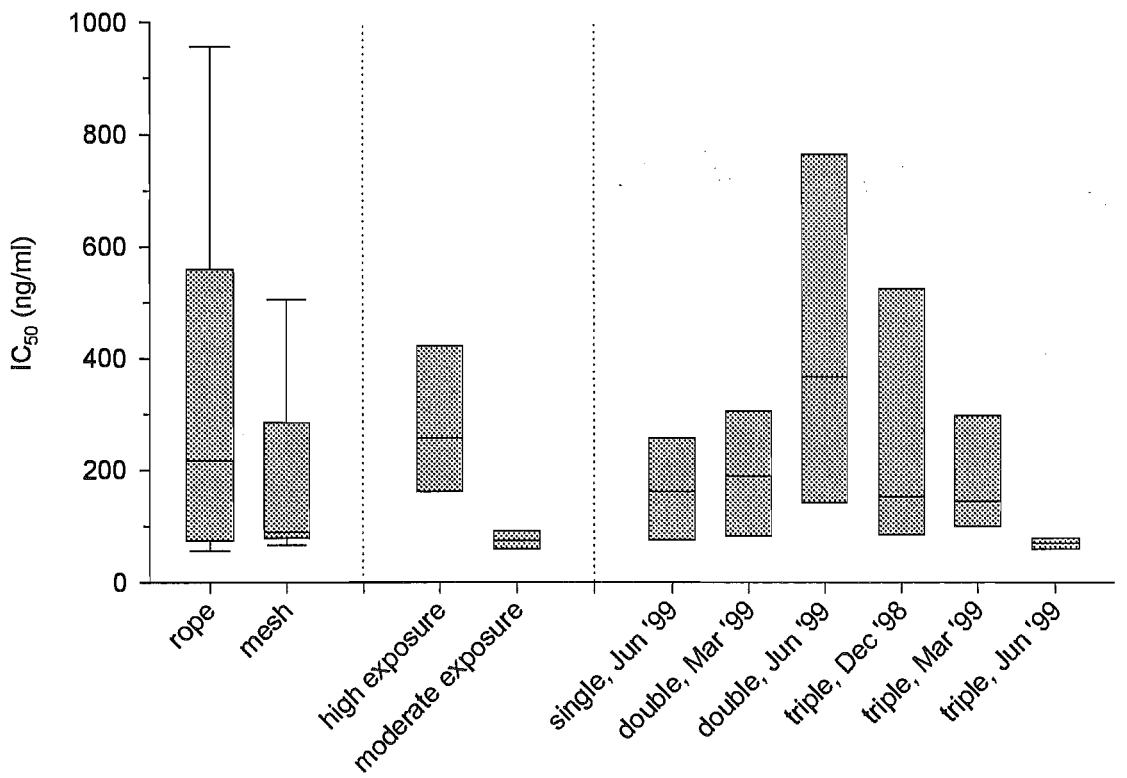


Figure 5.18. Final bioactivity (IC_{50}) of *P. croceus* between farming methods, exposures and harvest regimes. Harvest regimes further separated into harvest dates. Farming methods show the median, 5th and 95th percentiles (n=6 for each method). Both exposure and harvest regime show the range and median (n=4 for each treatment).

5.3.1.3. Fouling organisms

Both rope and mesh arrays were fouled with organisms. To compare the levels of biofouling between farming methods, thus indicating possible effects on sponge growth, the following was done using photographs taken in February '99. An acetate sheet with a grid pattern divided into $1cm^2$ squares was placed over each photo and the number of squares with ≥50% cover of fouling organisms ≤2cm from an explant, including over its surface, were countered. The percentage area of 2 dimensional space occupied by fouling organisms on and around ten explants (of *P. croceus*) farmed in each method were determined. The levels of biofouling differed greatly between the two farming methods (One Way ANOVA: $F_{df(1,18)}=102.12$, P=<0.00001), being on average, 61% (SE=15) on mesh arrays and 13% (SE=4) on rope arrays. Mesh arrays were also fouled with more types of organisms (Table 5.12).

Table 5.12. List of common fouling organisms found on the rope and mesh arrays.

Rope arrays	Mesh arrays
Bugula spp. (Bryozoa)	*Bugula* spp. (Bryozoa)
Aglaophenia sp. (Hydrozoa)	*Pyura rugata* (Ascidiacea)
	Perna canaliculus (Mollusca)
	Mytilus edulis (Mollusca)
	Aglaophenia sp. (Hydrozoa)
	small red seaweeds

Approximately 5% of *P. croceus* explants were also fouled with the hydroid *Aglaophenia* sp. These explants looked unhealthy, being yellow instead of the typical orange. Their pinacoderm was also pimply in texture and lacked the large papillae present on other explants. It is unknown whether *Aglaophenia* sp. caused this or settled on the explant after it became "sick". None of these explants were used in the bioassays. No organisms were observed fouling *L. brevis* explants.

5.3.2. Optimal farming density in the rope and mesh arrays

The results presented here concentrate on the effect that farming density has on the survival, final weights, and tissue yield per array of *L. brevis* and *P. croceus*.

5.3.2.1. *Latrunculia brevis*

Explant survival was similar between the three farming densities but differed greatly between the two farming structures (Table 5.13a). Overall survival was 51% in the mesh arrays and 11% in the rope arrays (Fig. 5.19a).

The high mortality of explants farmed in the rope arrays resulted in insufficient replicates to determine statistically in a full ANOVA model whether farming density affected final explant weights in rope arrays. Instead, the final weight data were pooled for both array types and compared between the three densities. This determined that farming density had no significant effect on the final weights of explants (Table 5.13b). However, final explant weight generally decreased as farming density increased (Fig. 5.19b).

The tissue yield per array was similar between the farming densities and structures (Table 5.13c). Because of poor explant survival and growth there was a net loss in weight for all treatments (Fig. 5.19c).

5.3.2.2. *Polymastia croceus*

Explant survival was similar across the three farming densities, but differed greatly between the two farming structures (Table 5.14a). Overall survival was 98% in the mesh arrays and 57% in the rope arrays (Fig. 5.20a).

The final weights of explants differed greatly between the farming densities (Table 5.14b) (Fig. 5.20b). Overall, the mean final weight of explants was 36g (SE=3.1) at the low density, 45.3g (SE=4.1) at the medium density, and 27.2g (SE=2.8) at the high density. Final weights were also greatly influenced by the farming structure (Table 5.14b). Overall, explants grew best in the rope arrays (Fig. 5.20b).

The tissue yield per array was similar between the farming densities and structures (Table 5.14c). All treatments produced a net gain in tissue weight except for explants farmed at high density on rope (Fig. 5.20c). Overall, net tissue gain was 35g per array.

Table 5.13. Analysis of variances of survival, final weights and tissue yield per array of *L. brevis* between farming methods and densities. GLM ANOVA used to analyse survival and tisssue yield data. One-Way ANOVA used to analyse weight data, pooled for density. To met assumptions, tissue yield data were log (+1) transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
a) survival					
method	1	18	18	10.45	0.007*
density	2	1.444	0.722	0.42	0.094
method*density	2	2.333	1.167	0.68	0.122
error	12	20.667	1.722		
total	17	42.444			
b) final weights					
density	2	1335.1	667.5	2.34	0.118
error	24	6858.9	285.8		
total	26	8193.9			
c) tissue yield					
method	1	2.714	2.714	3.71	0.078
density	2	0.363	0.181	0.25	0.784
method*density	2	0.131	0.0657	0.09	0.915
error	12	8.782	0.732		
total	17	11.989			

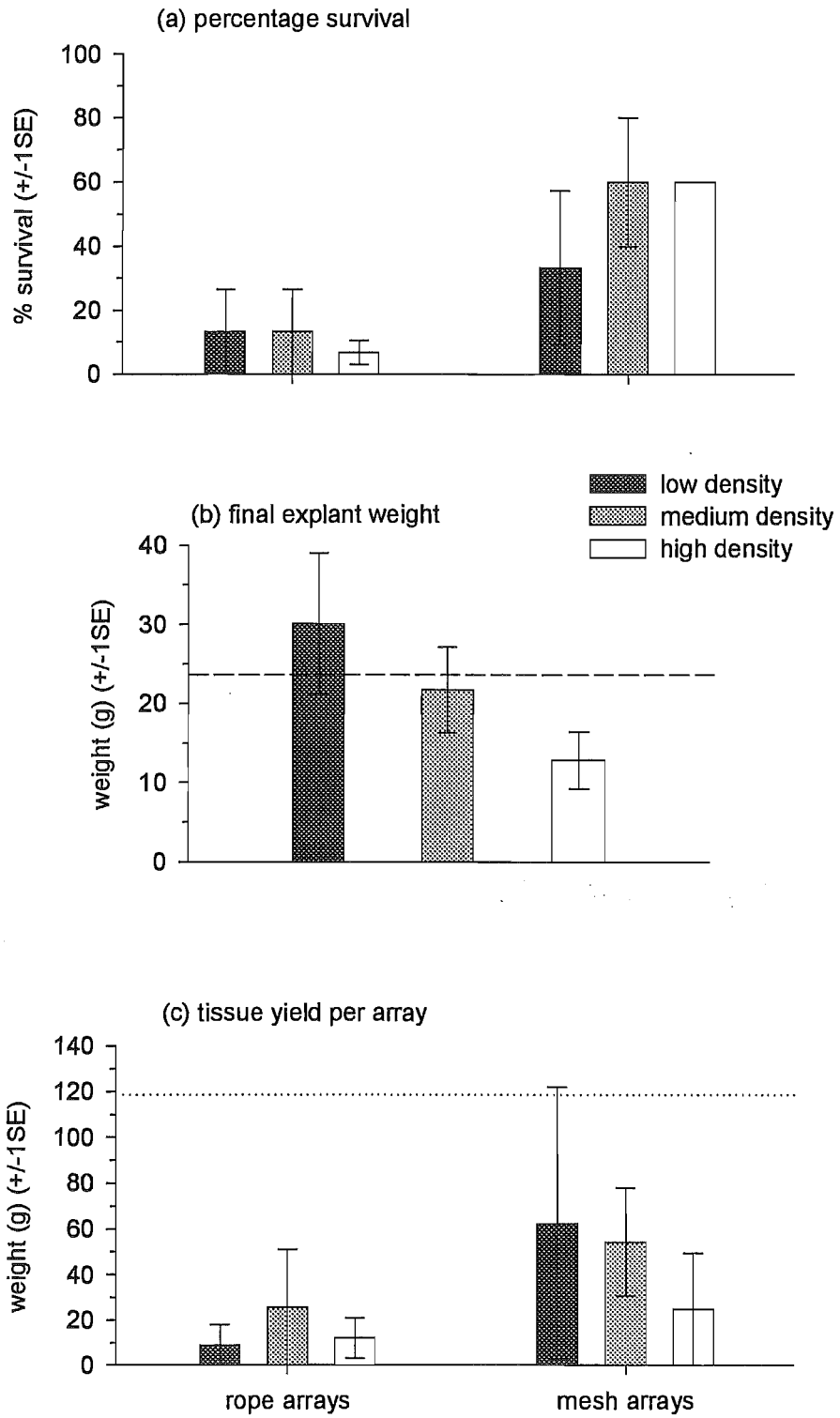


Figure 5.19. Mean percentage survival, explant weight and tissue yield per array of *L. brevis* farmed at different densities after 285 days. Survival and tissue yield further separated into rope and mesh arrays. Errors bars represent variation between arrays for survival and yield, and between explants for final weight. Dashed line represents mean initial explant weight. Dotted line represents mean initial tissue weight per array.

Table 5.14 Analysis of Variances of survival, final weights and tissue yield per array of *P. croceus* between farming methods and densities. GLM ANOVA used to analyse data. To met assumptions, survival data were arcsine transformed and weight data were log transformed. Prob: * = significant.

Factor	DF	SS	MS	F-ratio	Prob
a) survival					
method	1	2.631	2.631	23.39	0.0004*
density	2	0.0163	0.0082	0.07	0.931
method*density	2	0.197	0.0986	0.88	0.441
error	12	1.35	0.112		
total	17	4.194			
b) final weights					
method	1	0.336	0.336	8.53	0.004*
density	2	0.625	0.313	7.92	0.0008*
method*density	2	0.03	0.015	0.38	0.686
error	66	2.603	0.039		
total	71	3.527			
c) tissue yield					
method	1	841.9	841.9	0.22	0.649
density	2	5954.1	2977.1	0.77	0.484
method*density	2	542.6	271.3	0.07	0.933
error	12	46311.6	3859.3		
total	17	53650.2			

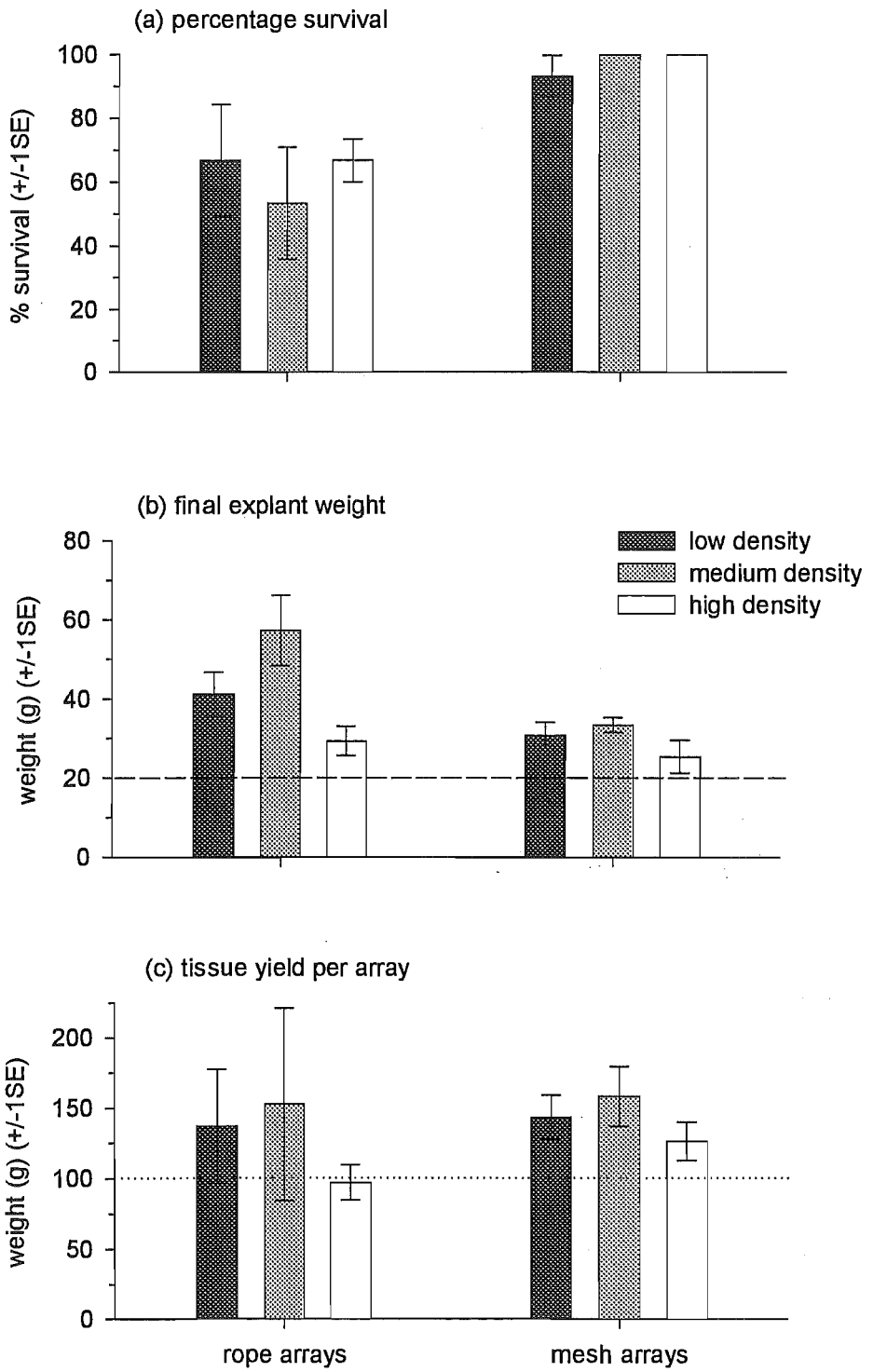


Figure 5.20. Mean percentage survival, explant weight and tissue yield per array of *P. croceus* farmed in rope and mesh arrays at different densities after 285 days. Errors bars represent variation between arrays for survival and yield, and between explants for final weight. Dashed line represents mean initial explant weight. Dotted line represents mean initial tissue weight per array.

5.3.3. Examining the commercial potential of rope and mesh arrays for other sponge species

Of the three species examined, *Mycale* sp. exhibited the greatest growth. Explants of *Mycale* sp. farmed in the mesh array grew from an initial volume of 27cm^3 to 2613cm^3 (SE=404) in 4 months (Fig. 5.21a). This final volume is a conservative estimate of size because over the farming period each explant grew several large lobes of tissue which broke away. All explants grew quickly through and over the mesh but did not fuse with neighbouring explants, and a distinct separation line, 1mm thick, could clearly be seen. After 1 month, all *Mycale* sp. explants farmed on the rope array had disappeared, probably, because they could not securely attach to the threaded PVA rope. In November `98 and February `99 a small amount of tissue was harvested to examine whether the farmed explants were producing the bioactive metabolites mycalamide and pateamine. Chemical analysis, done at the Marine Natural Products Laboratory at the Victoria University of Wellington, discovered that both metabolites were present at concentrations typically found in wild sponges (Table. 5.15). The wild sponges were sourced from Kapiti Island which has the closest known population of *Mycale* sp. to the farm location. Kapiti Island is ~50km from Wellington Harbour.

Table 5.15. Concentration of mycalamide and pateamine found in farmed and wild sponges of *Mycale* sp. in November `98 and February `99. Concentration given as milligrams of metabolite per kilogram dry weight of sponge.

	Wild sponges (mg/kg)	Farmed sponges (mg/kg)
Mycalamide, Nov `98	7-39	37
Mycalamide, Feb `99	8-68	18
Pateamine, Nov `98	2-36	13
Pateamine, Feb `99	0-82	24

The final survival of *Polymastia massilis* was good in both rope and mesh arrays, with only 1 explant in the mesh array dying after 4 months. The remaining 4 explants had not grown through the mesh and all 5 explants farmed in the rope array had attached to the threaded PVA rope. The final volumes of explants in the rope and mesh arrays were

similar (One Way ANOVA: $F_{df(1,7)}$=2.07, P=0.193), averaging 45.2cm^3 (SE=3.9) (Fig. 5.21b).

All explants of *Raspailia agminata* farmed in the rope and mesh arrays survived. Although the final volumes of explants were similar statistically between the farming methods (One Way ANOVA: $F_{df(1,8)}$=3.84, P=0.086), explants in the rope array were larger overall, being on average 43cm^3 (SE=6) compared with 29cm^3 (SE=4) in the mesh array (Fig. 5.21c). By February '99, 2 of the 5 explants farmed in the mesh array had grown partially through the mesh, while all 5 explants had attached to the threaded PVA rope.

5.4. Discussion

The farming responses of both *Latrunculia brevis* and *Polymastia croceus* differed greatly under the two farming regimes, with survival greatest in the mesh arrays and growth greatest in the rope arrays. The relatively poor growth of *L. brevis* and *P. croceus* in the mesh arrays probably resulted from several factors. One likely factor is that the mesh strands that surround and contain each explant covers ~11% of the explant's surface area and thus reduces its ability to feed, however, this problem is eliminated if the explant grows through the mesh.

The greater levels of biofouling on mesh arrays may have also reduced sponge growth. Biofouling is a serious problem in aquaculture and can limit the growth of the farmed organism either indirectly, by reducing water flow and thus food abundance (Paul and Davies 1986) or directly, through exploitative competition (Claereboudt et al. 1994). For exploitative competition to occur the diets of the fouling and farmed organism must overlap. Sponges feed primarily on ultraplankton (<10μm) (Reiswig 1971, 1975, Van de Vyver et al. 1990, Pile et al. 1996, 1997, Bell 1999). Ultraplankton is eaten by ascidians (Peterson et al. 1995, Ribes et al. 1998), bryozoans (Okamura 1992) and mussels (Lesser et al. 1992, Raby et al. 1997), which were all common fouling organisms on the mesh arrays. This suggests that fouling organisms could have reduced food abundance to the farmed explants. The levels of biofouling can vary greatly between farming locations (Claereboudt et al. 1994) and thus careful site selection may reduce biofouling on the mesh arrays.

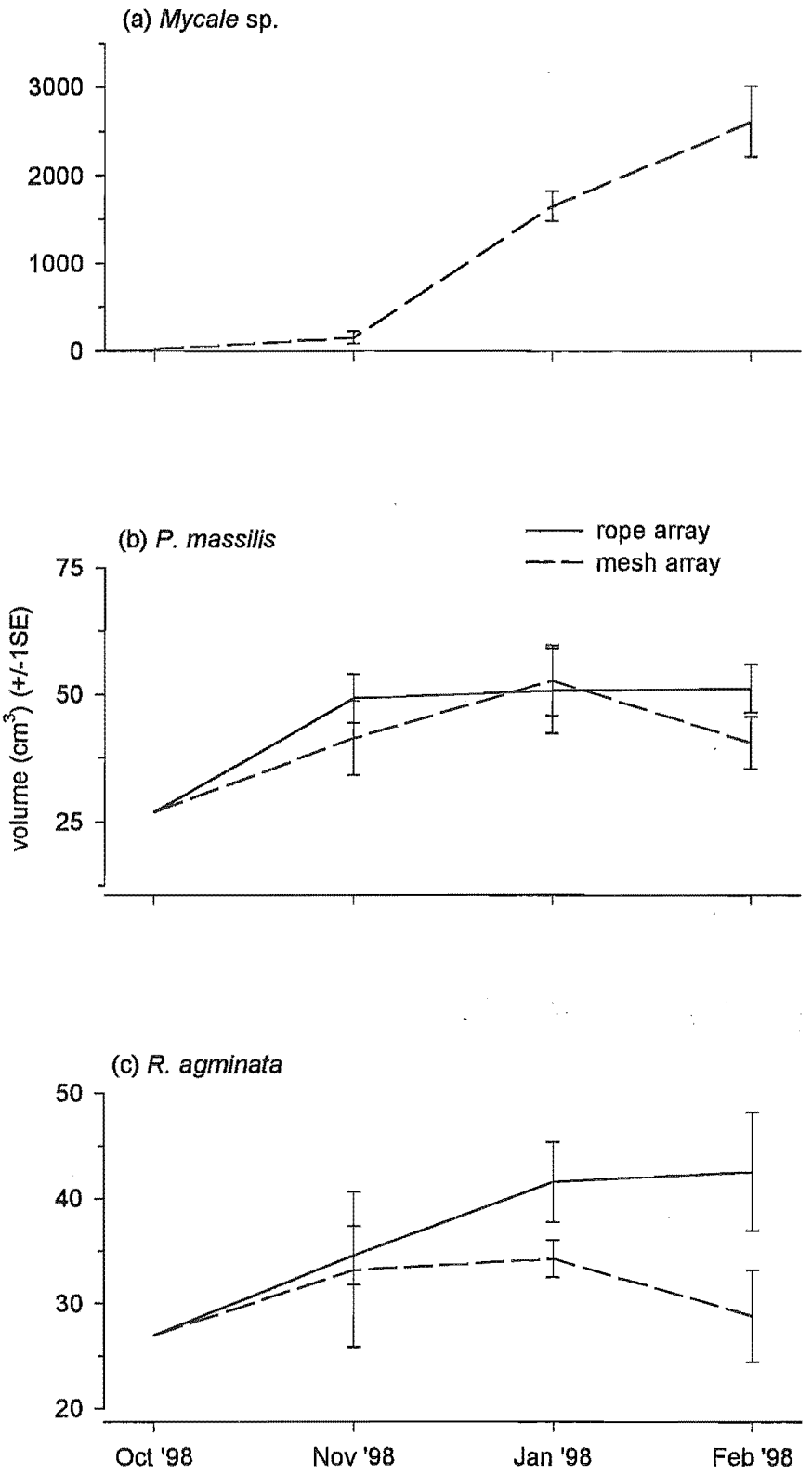


Figure 5.21. Mean volume of *Mycale* sp., *Polymastia massilis* and *Raspailia agminata* in the rope and mesh arrays from October '98 to February '99. All *Mycale* sp. farmed in the rope array had died by the first monitor. Error bars represent variation between explants.

Sponge growth may have also been reduced by the boundary layer created by each mesh array. The boundary layer is the layer of fluid adjacent to an object which has a mean velocity less than the surrounding fluid (Lincoln et al. 1998), and it can reduce the growth of both passive (Patterson 1984) and active suspension feeding invertebrates (Fréchette et al. 1989). The thickness of a boundary layer is determined by the shape and size of the object (Hollister et al. 1976) and since mesh arrays are larger than rope arrays they would therefore create a larger boundary layer around them. Therefore, the great difference in growth between explants farmed in mesh and rope arrays may be partially attributed to the difference in size of the boundary layer surrounding each array type. Because of their larger boundary layer, mesh arrays may have to be farmed with greater separation between them than rope arrays.

Growth in the mesh arrays is further reduced if explants are unable to grow out through the mesh strands, and thus restricting its final volume to the size of the mesh pocket. This ability to grow through mesh differs greatly between species and it may relate to tissue structure. The two species, *L. brevis* and *Mycale* sp., that grew rapidly through the mesh are relatively soft, fleshy sponges with low spicule density and unstructured mesohyl (Bergquist 1968, Bergquist and Fromont 1988, Kelly-Borges and Vacelet 1995). In contrast, species of the genera *Polymastia* have a well-developed choanosome, densely packed with spicules and separated into many layers (Kelly-Borges and Bergquist 1997) and no explants of *Polymastia massilis* and relatively few explants of *P. croceus* grew through the mesh strands. *Raspailia agminata* has firm but compressible tissue (Bergquist and Fromont 1988) and 2 of the 5 explants farmed in the mesh array grew partially through the mesh strands.

Although mesh arrays reduce growth, they promote explant survival. After nine months of farming, 96% of *P. croceus* and 61% of *L. brevis* survived in the mesh arrays but only 59% and 22%, repectively, in the rope arrays. The comparatively poor survival of explants farmed in the rope arrays may result from several factors. Explants suffer tissue damage when the rope is threaded through them and this can result in their death. In contrast, explants farmed in the mesh arrays are simply placed inside the mesh which causes them no damage. For sponges farmed in rope arrays, survival after threading differs greatly between species. For *L. brevis*, survival of explants decreased immediately after the rope was threaded which indicates a poor ability to survive tissue damage, while no *P.*

croceus explants in the rope arrays died in the first 3 months, indicating that it is not harmed by the threading process.

Survival also decreases when explants reject the threaded PVA rope as a suitable substrate for attachment, move away from it, and dislodge themselves. Attachment is also important for growth because explants of *L. brevis and P. croceus* that attached to the threaded PVA rope were heavier in final weight than explants that did not attach. Verdenal and Vacelet (1990) also discovered that explants of *Spongia officinalis* that attached to their fastening wire or identification tag had the highest growth rates. These studies suggest that to maximise growth and survival in rope arrays it is important to use a rope type (i.e. material and texture) that promotes explant attachment.

Another factor that can reduce survival in rope arrays is that large explants farmed in exposed waters can be pulled off the threaded rope. This was also recorded by Moore (1908a) and Duckworth et al. (1997) when they farmed sponges experimentally on threaded rope in exposed areas, and could be prevented by harvesting explants before they reach a large size.

These differences in the growth and survival of explants farmed in the two methods represent a trade-off that can affect the overall yield of sponge tissue. For example, growth of *L. brevis* was greatest in rope arrays but tissue yield was greatest in mesh arrays because this method promoted greatest survival.

For both *L. brevis* and *P. croceus*, harvested explants had similar or greater growth rates than non-harvested explants. This is a promising result for sponge aquaculture because it suggests that healing and regeneration of a new pinacoderm after harvesting does not divert energy away from overall somatic growth. This ability to regenerate lost tissue after damage has been observed in many sponges and is considered a survival mechanism to withstand partial predation, diseases, grazing and storms (Ayling 1981, Ayling 1983, Hoppe 1998, Battershill and Bergquist 1990).

Tissue harvesting clearly had an effect on the survival of *L. brevis*, because more single-harvested explants survived than double- and triple-harvested explants. However, the similar final survival between double- and triple-harvested explants indicated that repeat harvesting did not promote mortality. Instead, the water temperature at the time of harvesting appeared to be the most important factor because relatively more triple-harvest explants died after the March harvest, when the water temperature was 18°C, than after the December harvest when it was 16°C. The results of a previous experiment indicated

that a small temperature change of several degrees can greatly influence the survival of farmed *L. brevis* (Chapter 3). This study suggests that if *L. brevis* was farmed commercially then harvesting should occur in early summer, when water temperature is low, to promote explant survival. In contrast, survival of *P. croceus* was similar between the three harvest regimes, indicating that neither repeat harvesting nor the water temperature at the time of harvesting influenced post-harvest survival.

The exposure or degree of water movement can greatly affect the growth and tissue yield of farmed sponges. After nine months of farming, single-harvest explants of *L. brevis* were nearly three times heavier at the high exposure than at the moderate exposure. High water movement generally promotes high growth (Watson 1976, Wilkinson and Vacelet 1979, Verdenal and Vacelet 1990, Chapter 3) through increased availability of food. Because survival of *L. brevis* was similar between the two exposures, tissue yield had a similar pattern to final weight. The overall yield of tissue from the arrays situated at the high exposure was nearly three fold greater than from the arrays situated at the moderate exposure.

Unlike *L. brevis*, final weights of single-harvest *P. croceus* explants were similar between the two exposures. However, the volume of these explants in March '99, when they were at their maximum size, differed greatly between the two exposures, being nearly twice as large at the high exposure than at the moderate exposure. This indicates that growth of *P. croceus* explants is greatly affected by the interaction of exposure and time. This result supports an earlier study that found that growth of farmed *P. croceus* was greatest overall at the high exposure in spring (Chapter 3).

The growth of single-harvest explants of *L. brevis* and *P. croceus* varied between seasons, with explants growing during spring and summer and shrinking during autumn. Seasonal variation in growth is common in sponges and generally results from the interaction of water temperature and food abundance (Simpson 1968, Johnson 1979, Fell and Lewandrowski 1981, Barthel 1986, Turon et al. 1998). For both species in this study, however, the seasonal pattern of growth differed between farmed and wild sponges. For *P. croceus*, wild sponges peaked in size in January when the water temperature was greatest (Fig. 2.7) but farmed sponges continued growing for another two months to peak in size in March. In addition, wild sponges increased in volume by 70% over the farming period while single-harvest explants farmed in rope arrays grew by 270%. For *L. brevis*, wild sponges shrank by 40% of their initial volume while single-harvest explants farmed in rope

arrays at the high exposure grew by 530%. Maldonado and Young (1998) discovered that *Aplysina fistularis* and *Ircinia felix* grew quickest when transplanted to depths where their reproductive activity is suppressed. For several species of bivalves, farmed individuals grow better than wild individuals because of a greater energy allocation to somatic growth at the expense of reproduction (Rodhouse et al. 1984, MacDonald 1986). For *L. brevis* and *P. croceus*, the possible diversion of energy from reproduction into somatic growth may explain the different growth patterns between wild and farmed sponges. Regardless of the physiological mechanism, this study demonstrates clearly that farming can promote the growth of *L. brevis* and *P. croceus*.

However, growth can vary greatly between explants farmed in similar conditions. After nine months of farming, some single-harvest explants of *L. brevis* and *P. croceus* at the high exposure had shrunk by half of initial size while neighbouring explants had tripled in size. Such variable growth between explants farmed in similar conditions has been reported in many sponge species (Moore 1908a, Crawshay 1939, Thompson et al. 1987, Verdenal and Vacelet 1990, Duckworth et al. 1997, Osinga et al. 1999) and it may result from differences in initial explant health (Thompson et al. 1987) or handling procedure.

The farming density can also influence the growth of sponges. Explants of *L. brevis* and *P. croceus* were farmed experimentally at high (5cm separation), medium (10cm) and low (20cm) density. For *L. brevis*, final explant weights generally decreased as density increased. For *P. croceus*, final explant weights were significantly lower at high density than at the other densities. Poor growth at high density is common in aquaculture (e.g. Toro et al. 1995, Ramofafia et al. 1997) and generally results from reduced food availability.

For the commercial aquaculture of sponges to produce bioactive metabolites it is important to promote good production of sponge tissue and high biosynthesis of the target metabolite. In the main study, over half of the *P. croceus* farming treatments produced a net gain ≥50g per array. This represents at least a 50% increase in weight per explant over nine months. For *L. brevis*, single-harvest explants farmed in mesh arrays at the high exposure had a mean net gain of 80g per array. Tissue yield in the other farming treatments was poor with most recording a net loss of initial weight, resulting from poor explant survival. Therefore, good growth has to coincide with good survival otherwise poor tissue yield will result. Compared to other farming studies, both *L. brevis* and *P.*

croceus grew very well (Table 5.16). This comparison also indicates clearly that rope and mesh arrays are both very good farming structures for growing sponges.

Table 5.16. Comparison of growth rates, expressed as mean percentage increase per year, between sponge species farmed experimentally using different methods. This growth rate ignores any seasonal growth pattern. Codes: a = range over several farming sites, b = best farming site, c = high exposure

Species	Method	Growth rate
Hippiospongia and *Spongia* spp. (Moore 1908a)	attached to concrete discs	~100
Hippiospongia and *Spongia* spp. (Moore 1908a)	on threaded wire	~100
Hippiospongia lachne (Crawshay 1939)	attached to concrete discs	~150
Spongia agaracina (Verdenal and Vacelet 1990)	on threaded wire	38-90,a
Spongia officinalis (Verdenal and Vacelet 1990)	on threaded wire	0-150,a
Raspailia agminata (Duckworth et al. 1997)	in mesh bags	35,b
L. brevis	rope array	700,c
L. brevis	mesh array	270,c
P. croceus	rope array	360
P. croceus	mesh array	130

The second important requirement of commercial sponge aquaculture is to promote biosynthesis of the target bioactive metabolite(s). In June `99, farmed explants of *P. croceus* were a lot more bioactive (average IC_{50}=223) than wild sponges (IC_{50}=4250), suggesting that farming promotes bioactivity for *P. croceus*. For *L. brevis*, farmed explants (average IC_{50}=131) and wild sponges (IC_{50}=87) were both very bioactive. For *Mycale* sp., the concentrations of mycalamide and pateamine were similar between farmed explants and wild sponges. Such good metabolite biosynthesis from farmed sponges is not always expected. For example, farmed explants of the sponge *Lissodendoryx* n. sp. produced lower concentrations of the anticancer metabolite group Halichondrin than wild sponges (Munro et al. 1999). These results indicate that the effect of farming on metabolite biosynthesis, like growth and survival, can differ greatly between sponge species.

Overall, this study demonstrates that both rope and mesh arrays are good farming structures. However, each strcuture is most suited for a particular type of sponge, depending on its tissue structure. Rope arrays should be used to farm firm sponges such as

P. croceus, while mesh arrays are best for farming soft, fleshy sponges like *L. brevis* and *Mycale* sp. Explant bioactivity is very high in both rope and mesh arrays. Farming can produce high yields of sponge tissue. Therefore this study suggests that it possible to commercially farm sponges for their bioactive metabolites.

Chapter 6. Harvesting sponges from wild populations to supply bioactive metabolites

6.1. Introduction

Sponge harvesting to supply bath sponges dates back thousands of years to the Phoenicians and Egyptians (Storr 1957). Until recently, harvesting of bath sponges was an important and profitable industry for Mediterranean countries and for Florida State, USA (Kahn and Sandven 1946, Storr 1964, Verdenal and Verdenal 1987). However, overfishing and outbreaks of disease have recently devastated commercial sponge populations (de Laubenfels 1952, Storr 1964, Vacelet et al. 1994) and today the industry is much reduced and mostly confined to the Mediterranean (Vacelet 1985).

With the discovery of potentially new drugs from marine organisms, particularly the Porifera, the harvesting of sponges has once again become important. Sponges are now harvested in many countries to supply bioactive metabolites for drug discovery and development. Unfortunately, such harvesting is often environmentally destructive because many tonnes of a species may be removed to supply a few grams of metabolite (Anderson 1995). To prevent local population extinction and possibly loss of rare species, it is important to examine the effect of harvesting and find ways to lessen its destruction.

Harvesting, if managed properly, can provide a long term sustainable yield of target metabolites. For example, Page and Battershill (2000) examined the effect of dredging on the biomedically important sponge *Lissodendoryx* n. sp. They found that sponge biomass and density had both recovered to near original levels 7 months after harvesting, and estimated a sustainable annual harvest of 500kg. This is a biomass sufficient to supply metabolites for drug trials, but insufficient for full commercial harvest if the metabolite is used for drug production (Munro et al. 1999).

For common, fast-growing sponges, harvesting may provide the large biomass needed for extraction and drug manufacture. The harvesting method used will depend on where the sponge is found. For deep water sponges, dredging is the only available option but it is destructive and non-selective (Bergquist and Tizard 1969). It is also impractical over rocky reefs where the broken topography will damage equipment. For shallow water sponges, diving and hooking are also possible (Moore 1908b, Storr 1957, 1964, Bergquist

and Tizard 1969). Hooking is an old harvesting method where the fisherman working from a boat uses a claw attached to a long pole to hook and tear sponges from the substrate (Moore 1908b, Storr 1957, 1964). However, this method is only useful in clear water a few meters deep (Moore 1908b). For sponges that live on rocky reefs between depths of 5-50m the only possible method of harvesting them commercially is diving. This will likely involve divers swimming over reefs looking for the target species and removing a portion of each individual found. In the past, little regard was given to recovery of sponge populations and invariably whole sponges were completely removed.

Sustainable harvesting capitalises on the remarkably high regenerative ability of sponges to heal wounds and regrow lost tissue (Ayling 1981, 1983, Simpson 1984). However, many sponges have a threshold to recovery from damage, and if too much tissue is removed, by predators for example, the sponge will die (Dayton 1979, Shield and Witman 1993). It is therefore important to determine how much tissue can safely be removed for each species considered for harvesting. There is also a need to examine how quickly the sponge regrows harvested biomass, as this will determine how often a population can be harvested. The ability of sponges to regenerate lost tissue is considered a survival mechanism to withstand partial predation, disease, grazing and storms (Ayling 1981, Ayling 1983, Hoppe 1988, Battershill and Bergquist 1990).

This study examines the post-harvesting recovery of *Latrunculia brevis* and *Polymastia croceus* quantitatively, comparing growth and survival of individuals that have had either half, three-quarters or nearly all of their tissue removed. Sponges were harvested in spring, a season when survival of farmed *L. brevis* and *P. croceus* explants is high (Chapter 3). Bell (1998) has explored regrowth of *P. croceus* after harvesting but his results were inconclusive because of poor measuring techniques. This study will also demonstrate the effect on local *L. brevis* and *P. croceus* populations of removing sponges for farming experiments.

6.2. Methods

6.2.1. Experimental layout

For both *Latrunculia brevis* and *Polymastia croceus* the position of 15 sponges found close together at depths between 12-15m at Barrett Reef in Wellington (Fig. 1.2) were mapped. All sponges were medium size (about the size of a tennis ball) or larger. 15 sponges of each species were allocated randomly to one of three harvesting treatments: (1)

half of the sponge harvested; (2) three-quarters of the sponge harvested; and (3) all the sponge harvested, except for a thin basal remnant. Control sponges were five randomly selected sponges of each species in the same area which were monitored for other general ecological work (Chapter 2). Control sponges were of similar size to harvested sponges and were left intact.

6.2.2. Harvesting procedure

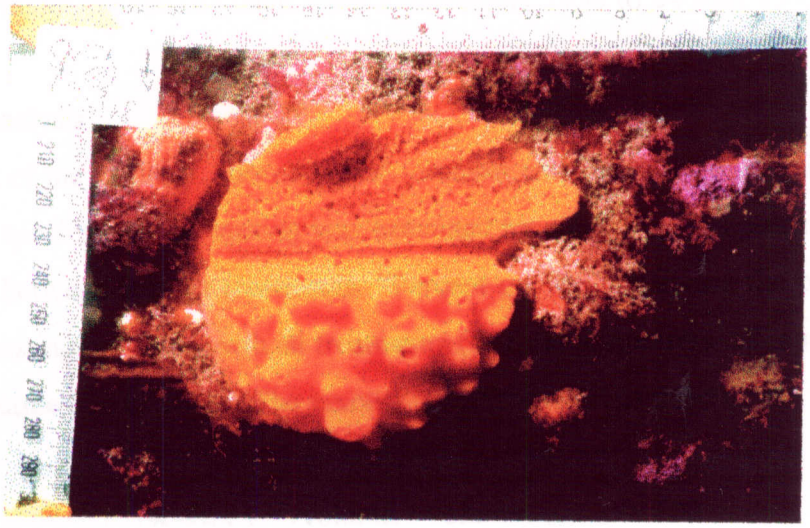
Using a sharp serrated knife tissue was harvested as follows. For sponges in treatments (1) and (2) a cut was made from the top of the sponge down through to the rock below, and then outwards leaving behind approximately a 5mm thick basal layer of sponge choanosome and basopinacoderm. One half or one quarter of the sponge was left intact, covered in pinacoderm (Fig. 6.1a,b). In treatment (3) sponges were cut parallel to their substrate removing all biomass except for a basal 5mm thick layer, showing exposed choanosome (Fig. 6.1c). The harvested biomass from each sponge was placed into individually labelled bags and weighed on return to the laboratory.

6.2.3. Monitoring growth and survival

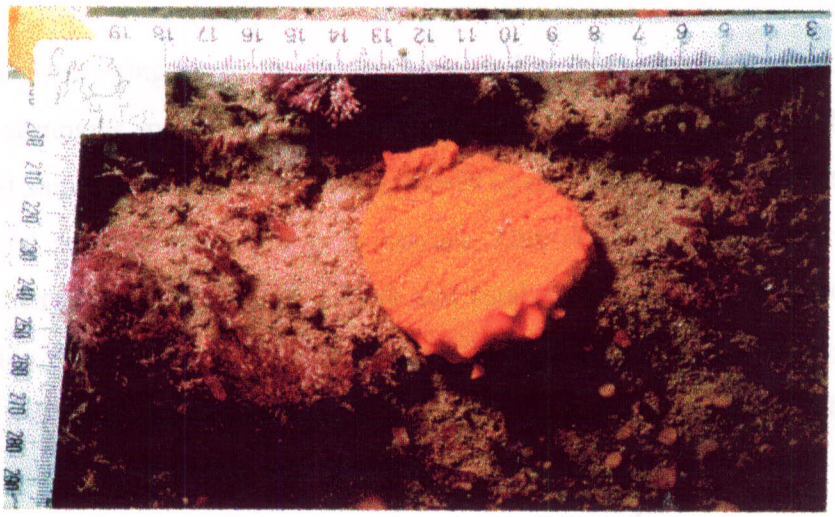
To examine the effect of harvesting *L. brevis* and *P. croceus* the cut sponges were monitored immediately before, immediately after, 1 week, 1 month, 2 months, 3 months, and 6 months after harvesting.

Determining the volume (cm^3) of each sponge *in situ* involved several steps. First, each sponge was photographed next to a ruler to provide a scale and the height of both cut and uncut portions was measured to the nearest 0.5cm. A frame attached to the front of the camera and pressed against the rock adjacent to the sponge ensured exact orientation of each photo. Next, the outline of each sponge, including cut basal regions, was traced onto acetate sheets. The trace was then digitised and the graphics programme OPTIMAS used to calculate the basal area (cm^2). Basal area and height were then multiplied to calculate the volume of each sponge. For treatments (1) and (2), adding the cut and uncut tissue volumes gave total sponge volume. The volume of inflated *P. croceus* individuals was multiplied by 0.63 to give a more accurate size estimate, as was previously used in the ecology experiment (Chapter 2). As well as size, survival and healing patterns, such as appearance of new oscules were recorded.

a) half harvested



b) three-quarter harvested



c) all harvested

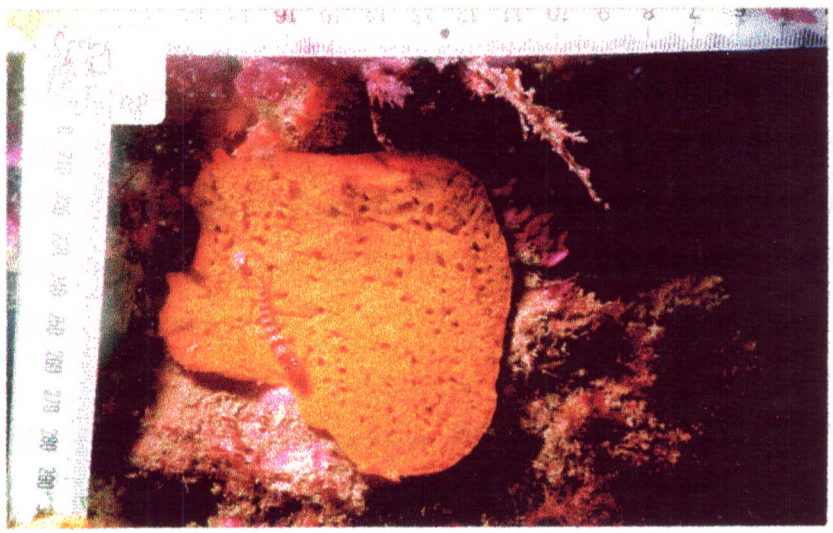


Figure 6.1. Photographs of the three harvest treatments used to examine the harvesting of wild sponges. Each photograph shows the exposed choanosome of *P. croceus* immediately after harvesting, rulers used for size measuring, and the descriptive label (top left corner).

This experiment ran for 203 days, from 2 November 1998 to 24 May 1999.

6.2.4. Statistical analysis

The effect of harvesting *L. brevis* and *P. croceus* was determined as follows. The regrowth of both species after harvesting was examined by comparing the percent growth and total growth between harvest treatments. "Percent growth" examines the percentage growth from harvesting onwards, with all treatments (half, three-quarters, all, and control) starting from the same size of 100%. The percent growth was determined by the formula:

$$\% \text{ growth at monitor t} = (\text{volume}_t / \text{volume}_{postharvest}) \times 100$$

where volume_t is the volume (cm^3) at monitor t and $\text{volume}_{postharvest}$ is the volume (cm^3) immediately after harvesting. Percent growth was used to compare regrowth over time. "Total growth" compares sponge volume (cm^3) immediately after harvesting to the volume (cm^3) at the final sampling date. This gives regrowth in cm^3 after 6 months. Total growth was for harvested sponges only. For each species, One-Way ANOVA's were used to determine statistically whether percent growth and total growth differed between harvest treatments. Finally, the recovery time for harvested sponges to grow back to original sizes in the 3 harvest treatments was estimated by projecting the 6 monthly growth rate for each species. The 6 monthly growth rate was determined by the formula:

$$\text{growth rate} = (\text{volume}_{final} - \text{volume}_{postharvest}) / \text{volume}_{postharvest}$$

where volume_{final} and $\text{volume}_{postharvest}$ are the volume of a sponge at the final sampling date and immediately after harvesting, respectively. The 6 monthly growth rate was used and not a mean monthly growth rate because the compound growth of each sponge would slightly distort the growth rate if calculated per month. Then, the recovery time in 6 month periods was determined by the formula:

$$n \text{ (6 month periods)} = \log (\text{volume}_{preharvest} / \text{volume}_{postharvest}) / \log (1 + \text{growth rate})$$

where $\text{volume}_{preharvest}$ and $\text{volume}_{postharvest}$ are the volume of a sponge immediately before and after harvesting, respectively.

6.3. Results

6.3.1. *Latrunculia brevis*

The initial volume of *L. brevis* ranged from 63 to 386cm^3 with a mean of 160cm^3 (SE=24). Initial volume did not differ significantly between harvest treatments (One-Way ANOVA: $F_{df(3,16)}$=0.37, P=0.78).

Because the initial volume within harvest treatments was so variable (Table 6.1) there was no significant difference in harvested weight between treatments (One-Way ANOVA: $F_{df(2,12)}$=0.51, P=0.62). The total weight across all replicates, however, differed between treatments (Table 6.1).

Table 6.1. Range of initial volume (cm^3) and total weight (g) of tissue harvested for *L. brevis* between harvest treatments.

Harvest treatment	Volume range (cm^3)	Weight (g)
half harvested	70-386	125.7
three-quarters harvested	63-339	151.8
all harvested	68-252	187.2

Percent growth at the final sampling date differed greatly between the 4 treatments ($F_{df(3,16)}$=12.52, P=0.0001), greatest for all-harvested *L. brevis* (Fig. 6.2a). These sponges increased in volume up to February `99, and then shrank slightly to be 3½ times their harvested size. *L. brevis* with either half or three-quarters of their biomass harvested also grew to February `99 (Fig. 6.2a). Percent growth was slightly better for the three-quarter harvested sponges. By May `99, control sponges had shrunk by half (Fig. 6.2a).

Unlike percent growth, total growth after harvesting was similar between the 3 harvest treatments ($F_{df(2,12)}$=0.37, P=0.696) (Fig. 6.2b). On average, *L. brevis* grew by 46.3cm^3 (SE=10.5) over the course of the experiment, or 0.23cm^3 per day. The 6 monthly growth rate for harvested *L. brevis* was 1.6 (SE=0.3). Growth was a process of filling in harvested biomass, with no sponges expanding laterally to invade new substrate. No sponge, six months after harvesting, had grown back to its pre-harvest size. The estimated time in years for *L. brevis* in each harvest treatment to grow back to their pre-harvest size, assuming a constant linear growth rate, was around 1 year (Table 6.2).

Table 6.2. Estimated recovery time in months for harvested tissue of *L. brevis* to grow back to original sizes in the 3 harvest treatments (±1SE).

Harvest treatment	Months
half harvested	8 (1)
three-quarters harvested	9 (1)
all harvested	14 (1)

All 15 harvested *L. brevis* survived which is a promising result considering the massive damage they incurred. Healing of cut tissue was rapid for *L. brevis*, with oscules appearing after 1 week. After 1 month, there were no obvious signs of the harvesting treatments apart from sponges being smaller. Healing was similar between the 3 harvest treatments.

One possibility was that harvesting would result in basal material regressing into smaller sponge clones. One *L. brevis* in the three-quarters harvest treatment broke up into three individuals after three months. However these fragments had rejoined into one individual by the end of the experiment. No buds were found around any harvested sponge.

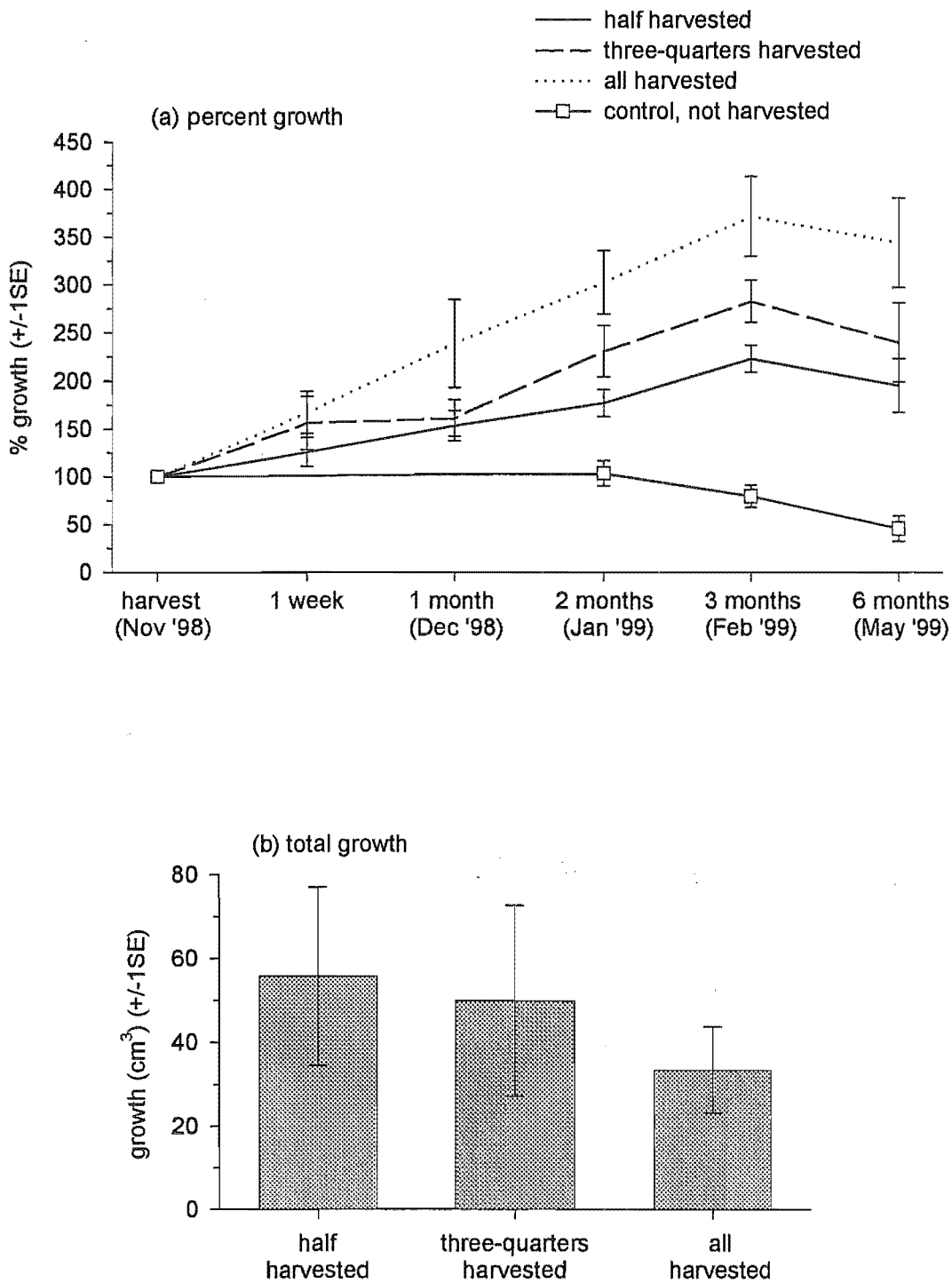


Figure 6.2. Mean percent growth and total growth of *L. brevis* after harvesting. For percent growth, control sponges were not measured 1 week and 1 month after harvesting. Error bars represent variation between sponges.

6.3.2. *Polymastia croceus*

The initial volume of *P. croceus* ranged from 35 to 620cm^3 with a mean of 182cm^3 (SE=30) and did not differ significantly between harvest treatments (One-Way ANOVA: $F_{df(3,16)}$=0.97, P=0.43).

As for *L. brevis*, the range of initial volumes of *P. croceus* within harvest treatments was so great (Table 6.3) that it obviated any differences in harvested weight between treatments (One-Way ANOVA: $F_{df(2,12)}$=1.45, P=0.27). However, the total weight of tissue harvested for *P. croceus* differed between treatments (Table 6.3).

Table 6.3. Range of initial volume (cm^3) and total weight (g) of tissue harvested for *P. croceus* between harvest treatments.

Harvest treatment	Volume range (cm^3)	Weight (g)
half harvested	60-174	115.2
three-quarters harvested	63-620	214.1
all harvested	35-460	292.9

The percent regrowth of *P. croceus* varied significantly between the 4 treatments ($F_{df(3,16)}$=3.77, P=0.032), greatest for all harvested sponges (Fig. 6.3a). By February '99 these sponges were three times their harvested size, but over the next 3 months they shrank by one third. *P. croceus* with three-quarters of their biomass harvested also grew up to February '99 and then shrank slightly over the next 3 months to be 20% larger than their harvested size (Fig. 6.3a). Percent growth of half-harvested *P. croceus* was poor with most sponges shrinking slightly after harvesting (Fig. 6.3a). After 6 months, the size of control sponges was unchanged (Fig. 6.3b).

Total growth of *P. croceus* after harvesting was similar between the 3 harvest treatments ($F_{df(2,12)}$=2.45, P=0.128) (Fig. 6.3b). On average, *P. croceus* grew by 5cm^3 (SE=4.5) or 0.024cm^3 per day. The 6 monthly growth rate for harvested *P. croceus* was 0.36 (SE=0.16). Growth was a process of filling in harvested biomass, with no sponges expanding laterally to invade new substrate. No sponge, six months after harvesting, grew back to its pre-harvest size. The estimated time in years for *P. croceus* in each harvest treatment to grow back to pre-harvest size, assuming a constant linear growth rate, was around 1½-4years (Table 6.4).

Table 6.4. Estimated recovery time in months for harvested tissue of *P. croceus* to grow back to original sizes in the 3 harvest treatments (±1SE). Calculation for "half harvested" treatment assumes they stopped shrinking in size and regrew.

Harvest treatment	Months
half harvested	17 (3)
three-quarters harvested	26 (3)
all harvested	45 (4)

As for *L. brevis*, all 15 harvested *P. croceus* sponges survived. However, healing of *P. croceus* after harvesting was slower than in *L. brevis*. For *P. croceus*, oscules appeared after one month and all sponges looked fully healed after three months. Many sponges healed in an interesting fashion, with their uncut tissue curling over their cut tissue, as in appearance like a surf wave. This was also observed by Bell (1998) studying *P. croceus* at Leigh. Healing was similar between harvest treatments, and no buds were found around any harvested sponge.

6.4. Discussion

Based on the above results, harvesting wild populations of *Latrunculia brevis* and *Polymastia croceus* may supply sufficient and sustainable quantities of bioactive metabolites needed for commercial drug manufacture. After harvesting, *L. brevis* grows quickly and, assuming a constant growth rate, it would take about 1 year for sponges to replace harvested tissue. In comparison, harvested *P. croceus* grew more slowly but could replace harvested tissue within 4 years. These recovery times assume a constant linear growth rate which oversimplifies the growth dynamics of both species. An ecological study discovered that growth of *L. brevis* and *P. croceus* varies over years and seasons with generally high growth during winter and spring, and poor growth during summer and autumn. The 6 monthly growth rate used to calculate recovery times included the summer and autumn periods. This may suggest that the recovery times after harvesting presented here are conservative. *L. brevis* can live for at least 3 years (Chapter 2) while *P. croceus* can live for over 15 years (Ayling 1976) so it may be possible to harvest the same sponge on several occasions. Repeat harvesting on explants of *L. brevis* and *P. croceus* did not negatively affect their regrowth (Chapter 5).

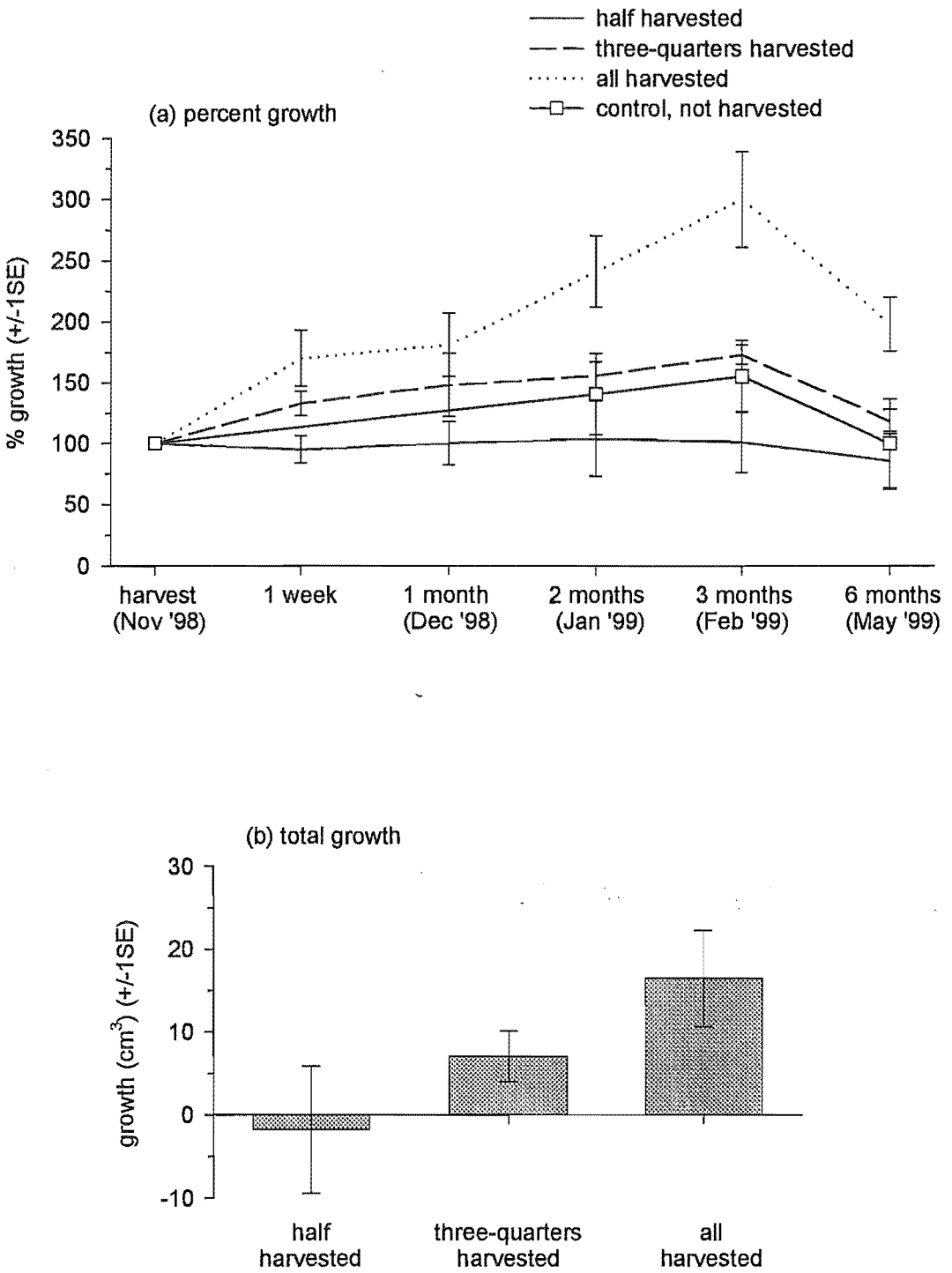


Figure 6.3. Mean percent growth and total growth of *P. croceus* after harvesting. For percent growth, control sponges were not measured 1 week and 1 month after harvesting. Error bars represent variation between sponges.

The different growth rates of *L. brevis* and *P. croceus* after harvesting may result from differences in choanosome structure. The choanosome in species of *Latrunculia* is unorganised, consisting of an irregular reticulation of spicule fibres (Bergquist 1968, Kelly-Borges and Vacelet 1995). In contrast, *Polymastia* species have a well-developed choanosome, densely packed with spicules and differentiated into many layers (Kelly-Borges and Bergquist 1997). Simpson (1984) suggested that the extra cellular investment required to heal and repair wounds in sponges with a structured choanosome may reduce their growth.

Of the two species investigated, *L. brevis* is a better candidate for commercial harvesting because it grows more quickly after harvesting. Comparing growth between the harvest treatments, shows some harvested sponges trebled in size, while non-harvested sponges shrank by half. Rapid growth after damage, as seen in *L. brevis* and to a lesser extent in *P. croceus*, has not been recorded before in other massive, discrete sponges, where the normal response is wound regeneration only. For example, Storr (1964) studying *Hippiospongia lachne* and *Spongia barbara*, observed regeneration of ectosome tissue in these sponges following experimental damage, but no subsequent growth of new biomass after 3 months. While Hoppe (1988) discovered that *Neofibularia nolitangere* and *Ircinia strobilina* take from 1-5 months to repair and fill in small 9cm^3 lesions cut into their ectosome. However, cutting did not trigger growth of new biomass. Another species, *Agelas clathrodes*, which he also studied had not filled in the lesion depression after 20 weeks.

It is important to separate regeneration and growth processes in sponges (Reiswig 1973, Jackson and Palumbi 1979, Hoppe 1998). Regeneration involves the reorganisation of existing cells (Korotkova 1963, Simpson 1984) and can quickly regain coverage of primary substratum (Ayling 1981, Ayling 1983). Growth, in comparison, involves the formation of new cells and skeletal material, and leads to an increase in size (Simpson 1984). A >200% mean increase in size of "all harvested" *L. brevis* and *P. croceus* after 3 months indicates that both species were producing new biomass and "growing". The formation of new oscules after one week for *L. brevis* and 1 month for *P. croceus* suggests a rapid reorganisation of their damaged canal system, and this may have allowed harvested sponges to restore normal feeding and to grow at maximum rate.

The similar total growth between harvest treatments for both species indicates that the differences in percent growth between all harvest treatments are an artefact of their

different post-harvested size. This is not surprising because wild sponges of *L. brevis* and *P. croceus* grew at a similar rate irrespective of their size (Chapter 2). Varying percent growth over time suggests that, like growth of wild sponges, regrowth of harvested sponges is influenced by the seasonal variation in water temperature and its effect on food availability.

The fact that no sponges of either species died, even after most of a sponge was removed, further illustrates the ability of sponges to survive damage and heal wounds. The healing rates of *L. brevis* (<1 month) and *P. croceus* (<3 months) are similar to those observed in other massive, discrete sponges. For example, the rate of healing to repair wounds was 5 months for *Rhabdocalyptus dawsoni* (Leys and Lauzon 1998) and between 1-5 months for *Neofibularia nolitangere* and *Ircinia strobilina* (Hoppe 1988). The unusual healing action of *P. croceus* where the ectosome adjacent to the cut area curls over and covers the wound has been observed in other Demospongiae, (*Tethya lyncurium*), and was suggested by Connes (1996) to result from the well-developed cortex of the species, a feature shared by *P. croceus*.

Individual recovery, or growth, survival and regeneration, after harvesting are some of the major factors that determine whether a sponge species is suitable for commercial harvesting. Considering individual recovery alone, we can speculate on the harvesting potential of both *L. brevis* and *P. croceus*. If we assume constant linear growth rate, zero mortality and a population density of 1 medium-sized individual per $25m^2$ (a conservative density for both species on exposed subtidal rocky reefs around Wellington) we can expect to harvest, per square kilometre of subtidal rocky reef, 150kg of *L. brevis* every year and 234kg of *P. croceus* every 4 years. These harvesting figures do not allow for variation in sponge growth and survival between years which will influence tissue yield.

This study has shown that small-scale harvesting to supply tissue for farming trials or metabolite for drug development will have virtually no impact on *L. brevis* populations as harvested sponges will quickly regrow harvested tissue. Small-scale harvesting will have a greater effect on *P. croceus*, however, the population biomass should return to an original level within 4 years. Overall, this study suggests that the commercial harvesting of wild populations is possible for at least two sponge species. However, this option may be limited because of the relative scarcity of the sponges in their natural environments.

Chapter 7. General Discussion

Bioactive metabolites from sponges have great potential in the pharmaceutical industry as drugs or biomedical tools. However, there are frequently problems associated with supplying the large quantities of sponge metabolites that may be required commercially. Indeed supply of even small quantities for drug development can be difficult. Of the supply methods being examined currently, aquaculture is considered to be the most cost-effective and in the medium term the only method to guarantee sufficient supplies of some sponge metabolites (Shimizu 1995, Munro et al. 1999). This study developed techniques for the aquaculture of sponges for metabolite production, which are potentially applicable to other soft-bodied marine organisms. It focused on two species, *Latrunculia brevis* and *Polymastia croceus*, that contain bioactive metabolites of interest to the pharmaceutical industry. This study had two main aims:

(1) to examine how the environment affects the growth, survival and metabolite biosynthesis of sponges and,

(2) to develop farming structures that are suitable for the large-scale commercial aquaculture of sponges for metabolite production.

These objectives were achieved by a combination of *in situ* survey and experiments. The ecology of *L. brevis* and *P. croceus* was examined over several years to determine the seasonal effects on the growth, survival and metabolite biosynthesis of wild sponges. Short-term transplant experiments explored the effect of the season of out-planting and relative exposure on the responses of sponges to farming situations. Sponge growth and survival in response to different farming methods and materials were examined to help develop structures suitable for sponge aquaculture. The environmental effect on growth, regeneration and survival of sponges farmed using different techniques was further explored in a separate experiment. Lastly, the effect of harvesting on growth, regeneration and survival of wild sponges was examined to determine whether harvesting wild populations is an alternative and commercially viable method of supplying bioactive metabolites.

Although sponges are an important component in many reef habitats (Reiswig 1973, Bergquist 1978, Scubauer et al. 1990), many aspects of their biology are poorly understood. In this final chapter, the common relationships between the separate parts of

this study to develop our understanding of how the environment affects the growth, survival and metabolite biosynthesis of sponges both in nature and in artificial conditions within farms are examined.

7.1. Growth of sponges

Growth of sponges, as reviewed by Simpson (1984), involves several developmental stages. First there is the initial formation of new cells and functional areas such as choanocyte chambers, then the development of skeletal structures, followed by the remodelling of the mature tissue and canal system to optimise water flow and feeding, which all lead to an increase in size or growth. This study, like most others, did not separate these development stages but considered instead overall growth only, measured as a change of volume (cm^3) or weight (g).

Given the importance of sponges in many benthic communities (Dayton et al. 1974, Bergquist 1978, Schmahl 1990), there are surprisingly few longer-term field studies examining what environmental factors influence their growth. However, several studies have found a seasonal pattern of growth for some temperate and tropical sponge species. For example, Stone (1970) found that the temperate, intertidal sponge *Hymeniacidon perleve* grows during summer and shrinks during winter, while Reiswig (1973) observed that the Caribbean sponge *Mycale* sp. grows quickest in winter and spring. The lateral growth of the encrusting sponge *Haliclona permollis* was greatest in summer and autumn (Elvin 1976). Fell and Lewandrowski (1981) found that an unidentified species of the genus *Halichondria* grows during spring as water temperature rises, while Barthel (1986) observed that *Halichondria panicea* grows in spring and early summer and shrinks in autumn. Finally, the common Mediterranean species *Crambe crambe* grows fastest in spring and summer as water temperature rises (Turon et al. 1998). This study monitored growth of wild *L. brevis* and *P. croceus* over two years and found that although there was some individual and annual variation, sponges generally grew during winter and spring as water temperatures rose and shrank during summer and autumn as water temperatures fell. These seasonal patterns of growth were also observed in two transplant experiments which examined the seasonal and exposure effects on the farming responses of *L. brevis* and *P. croceus*.

Although the season or seasons of maximum growth may differ slightly between species, the overall pattern of growth indicated by these studies is that sponges generally

grow as the water temperature rises and shrink as the water temperature falls, and it may relate to seasonal variation in food abundance and reproductive investment. Sponges feed primarily on ultraplankton (<10μm) (Reiswig 1971a, 1975, Van de Vyver et al. 1990, Pile et al. 1996, 1997, Bell et al. 1999), which generally increase in abundance as water temperature rises to peak in density in summer (Fogg 1986, Joint 1986, Waterbury et al. 1986, Tamigneaux et al. 1995). Falling food abundance probably accounts for many sponges shrinking after summer but for some species reproductive investment may also be a factor. Both Elvin (1979) and Barthel (1986) studying *Haliclona permollis* and *Halichondria panicea*, respectively, suggested that adult sponges degenerate after reproduction in summer. In this study, *P. croceus* mainly recruited in autumn which agrees with Ayling (1980) who found that it is reproductively active in summer and early autumn only. Therefore, its seasonal patterns of growth may also result from seasonal variation in reproductive investment. For some species, however, reproductive investment does not account for seasonal growth patterns. Turon et al. (1998) found that small sponges of *Crambe crambe* showing no reproductive activity also vary in size between seasons. Recruitment of *L. brevis* was found to occur in all seasons suggesting that it is reproductively active throughout the year and, therefore, its seasonal growth patterns are unlikely to result from seasonal variation in reproductive investment. These studies indicate that seasonal variation of growth in sponges results from the interaction of water temperature, food abundance and reproductive investment, with the relative importance of each factor varying with species, seasons and possibly latitude.

In contrast to the above examples, some temperate and tropical sponge species show no seasonal pattern of growth (Ayling 1983, Hoppe 1988, Pansini and Pronzato 1990). While this may reflect a stable, unchanging environment, it is interesting to note that the sponges in these studies grew very slowly and it is possible, therefore, that any seasonal growth patterns were below the resolution threshold of their measuring techniques. In addition, the lateral growth of the thin encrusting sponge species studied by Ayling (1983) may have been obstructed by interactions with neighbouring organisms and this may have also prevented seasonal growth.

The supply of food to, and subsequent growth, of sessile suspension feeding invertebrates is affected not just by food abundance, but also by the ambient flow of water (Patterson 1984, Best 1988, Sebens and Johnson 1991, Helmuth and Sebens 1993, Kim and Lasker 1998). Although sponges are active suspension feeders and capable of

generating their own water movement, studies by Reiswig (1971b) and Vogel (1974) suggest that the rate at which sponges pump water remains constant regardless of the ambient water flow, thus indicating that sponges do not compensate in areas of low water flow by pumping harder. Therefore, the supply of food within each season and the subsequent growth of a sponge is affected primarily by the ambient water flow or exposure. This indicates that growth of sponges should vary between locations differing in the degree of exposure. Apart from the preliminary study by Watson (1976), who found that individuals of *Ancorina corticata* were larger overall in an exposed area than at neighbouring, more sheltered areas, this relationship between growth and exposure has not been studied in wild sponges. Manipulative studies where sponges are transplanted to different exposure regimes are probably the best way to examine this relationship, but studies are surprisingly few and have produced conflicting results. While Wilkinson and Vacelet (1979) observed that growth of transplanted sponges generally increased as exposure increased, Duckworth et al. (1997) and Leichter and Witman (1997) observed that growth of sponges in their studies was poorest overall in the most exposed treatments. The present study examined the relationship between sponge growth and exposure in two transplant experiments. In the first experiment, explants of *L. brevis* and *P. croceus* were grown for two months in each season at different depths at three sites differing in exposure (low, moderate and high). In the second experiment, explants were grown for nine months at the moderate and high exposure only. Although there was some variation between seasons, growth of sponges was observed generally to increase with exposure.

The results of this study also suggest why some sponges have relatively poor growth in very exposed conditions. In the first experiment, growth at the low and moderate exposure sites was greatest at the shallowest depth of 5m, but at the high exposure site growth was greatest at a depth of 10m. An experiment examining the erosion of plaster-of-paris discs determined that water movement was greatest overall at the depth of 5m at the high exposure site. In addition, dive surveys determined that both species occur naturally at the high exposure site only and below a depth of 7m. The results from the two sheltered sites indicates that high light intensity at the depth of 5m does not negatively affect the growth of either *L. brevis* or *P. croceus*. Therefore, the very high water movement at a depth of 5m at the high exposure site, an exposure regime outside their normal range, most likely caused the poor growth of *L. brevis* and *P. croceus*. The work of Palumbi (1984, 1986) indicates the mechanism behind this poor growth. The

canal diameter of *Halichondria panicea* (the species studied also by Leichter and Witman (1997)) is smaller in sponges living in exposed waters than in more sheltered waters. This increases the cost of pumping water for sponges living in exposed conditions and reduces their growth. The relationship between sponge growth and exposure indicated by these studies is that growth will generally increase with increasing exposure until the cost of high water movement (in terms of pumping) outweighs the benefit of supplying more food.

In addition to supplying food, the flow of water also transports sediment which settles predominantly in sheltered areas because of low turbulence. Verdenal and Vacelet (1990) and Duckworth et al. (1997) found that sponges smothered by sediment have reduced growth. Therefore, another environmental reason why sponges transplanted to sheltered locations have poor growth may result from the greater incidence of smothering by sediment.

The great effect of the environment on the growth of *L. brevis* and *P. croceus* demonstrates clearly the indeterminate growth of sponges. Indeterminate growth implies that growth and final size of an organism is determined more by environmental conditions such as exposure than by its genetics (Sebens 1987). However, final size is not fixed and if conditions should deteriorate so that food capture is insufficient for the metabolic demands of the organism, then it may shrink. This is most commonly seen in clonal or modular organisms such as sponges and sea anemones where food capture and metabolic cost is proportional to the mass of each module (Sebens 1987). Indeterminate growth of sponges, where size and growth vary between seasons, years and habitats, makes estimation of their life spans difficult if not impossible.

One interesting feature of sponges is that they can vary greatly in growth, with some sponges growing well while neighbouring sponges shrink (Storr 1964, Reiswig 1973, Dayton 1979, Fell and Lewandroski 1981, Ayling 1983, Hoppe 1988, Wulff 1991, Leys and Lauzon 1998). This was observed for wild *L. brevis* and *P. croceus*. Variable growth is also common between explants farmed in seemingly similar environmental conditions (Moore 1908, Crawshay 1939, Thompson et al. 1987, Verdenal and Vacelet 1990, Duckworth et al. 1997, Osinga et al. 1999). In the present study, for example, some single-harvest explants of *L. brevis* and *P. croceus* farmed at the high exposure for nine months had shrunk by half of their initial size while neighbouring explants had tripled in size. Variable growth in sponges may result from several factors, Johnson (1979) suggested that intraspecific competition for food between neighbouring individuals of *Clathrina blanca*

resulted in some growing well while others shrank. Differences in reproductive activity between sponges is common (Ayling 1980) and because reproduction is considered a drain on resources (Sebens 1987), particularly so for sponges where gamatogenesis involves the transformation of choanocytes (feeding cells) into sperm sex cells (Simpson 1984), it may also promote variation in growth between individuals. Thompson et al. (1987) discovered that genetically identical explants of *Rhopaloeides odaribile* varied greatly in growth and suggested that it may result from differences in initial explant health or nutritional stores. In addition, genetic differences between sponges or explants could also promote variation in growth. Minor differences in the handling procedure when cutting sponges and transplanting explants may have also induced large differences in growth. However, the good survival of *P. croceus* after transplanting indicates that any minor differences in handling procedure were unlikely to have promoted variation in its growth. Although all organisms show variation in growth between individuals, these many ecological and farming studies indicate that sponges show exceptionally great variation.

There is a general perception that sponges grow slowly (Reiswig 1973, Dayton 1979, Ayling 1983, Hoppe 1988, Pansini and Pronzato 1990, Leys and Lauzon 1998, Turon et al. 1998). For example, of the 10 species studied by Dayton (1979) only 1 species (*Mycale* sp.) had noticeably grown after 10 years. Leys and Lauzon (1998) found that the average growth of 19 individuals of *Rhabdocalyptus dawsoni* was 2cm per year. In this study, wild sponges of *L. brevis* and *P. croceus* after 2 years had, on average, halved and doubled in size, respectively. However, farming and harvesting experiments demonstrated that sponges can have very high growth rates. For example, single-harvest explants of *L. brevis* and *P. croceus* farmed in rope arrays at the high exposure had grown by an average of 950% and 740% of their initial volume, respectively, in six months. Most harvested sponges of both species that had ≥90% of their tissue removed grew by ~200% of their post-harvested size in 3 months. Before growth is possible the sponge has to first heal and reorganise its damaged canal system. Regeneration is fast in some sponges (Jackson and Palumbi 1979) including *L. brevis* and *P. croceus* and it involves the reorganisation of existing cells (Korotkova 1963, Simpson 1984). Regeneration does not, however, lead to an increase in size. In comparison, growth involves the formation of new cells and skeletal material (Simpson 1984) and the great increase in sponge size in this study indicated that both farmed and harvested sponges were producing new tissue and growing.

What factors may have promoted the good growth of farmed and harvested *L. brevis* and *P. croceus*? Active suspension feeders suspended in the water column grow quicker than conspecifics on the substrate and in the benthic boundary layer because of a greater availability of food (Fréchette and Bourget 1985). Farmed sponges in this study were grown in the water column and, therefore, would have had greater access to food compared to wild sponges. In addition, farmed sponges were surrounded by fewer encroaching and competing organisms and this would have also resulted in greater food availability. Although these factors probably promoted the growth of farmed sponges, they cannot explain why harvested sponges, where the ambient environment (water movement and competitors) remained constant, also had greater percentage growth than neighbouring non-harvested sponges. One factor that may have increased growth in harvested sponges is the diversion of energy away from reproductive activity, which is a drain on resources (Simpson 1984, Sebens 1987), and into somatic growth. However, sponges in one harvest treatment had ≥90% of their tissue removed and it is difficult to imagine that these sponges could triple in size in 3 months solely by energy diversion from their remaining 10% of their tissue. Therefore the greater growth of harvested, and farmed explants, compared with wild sponges may indicate that the act of tissue damage, either from harvesting tissue or from cutting sponges to make explants, promotes growth in *L. brevis* and *P. croceus*. Greater relative growth of damaged individuals compared with non-damaged individuals has not, as far as the author knows, been found in other sponges or marine invertebrates and thus it requires further study to establish the nature of the effect.

7.2. Survival of sponges

Except for short-lived species that have high mortality around summer (Johnson 1979, Fell and Lewandroski 1981, Frost et al. 1982), adult sponges of most species, including *L. brevis* and *P. croceus*, show no seasonal pattern of survival (Reiswig 1973, Dayton 1979, Turon et al. 1998). Although seasonal cycles of water temperature had no effect on the survival of wild *L. brevis* and *P. croceus*, the survival of farmed *L. brevis* explants varied greatly between the farming seasons. In an experiment that farmed *L. brevis* for 2 months in each season, survival was greatest in winter when the water temperature was lowest (9°C). In a separate experiment, explants harvested in December '98 when the water temperature was 16°C survived better than explants harvested in

March '99 when the water temperature was higher (18°C). These results indicate that cooler water increases the ability of *L. brevis* to survive transplanting and reorganise its cut tissue into fully functional explants, and it may result from several factors. Respiration is lower in cooler water (Barthel and Theede 1986, Burlando et al. 1992, Cheshire et al. 1995) which reduces stress during transplanting. Cooler water also promotes quicker pinacoderm healing (Duckworth et al. 1997) and reduces microbial growth (Hummel et al. 1988, Vacelet et al. 1994), both of these effects may reduce the chance of infection.

The seasonal variation in the ability of *L. brevis* to heal wounds and survive is an interesting finding for sponge ecology, particularly in how it relates to the survival of sponges after partial predation. Many sponges are eaten by fish, asteroids or echinoids (Dayton et al. 1974, Ayling 1978, Keough and Butler 1979, Shield and Witman 1993, Wulff 1995, Dunlap and Pawlik 1996). Although these organisms may remove only part of an individual (i.e. partial predation), many sponges have a threshold to recovery from damage and if too much tissue is eaten the individual will die (Dayton et al. 1974, Shield and Witman 1993). Although this study found no evidence of predation on *L. brevis*, the survival results of farmed *L. brevis* suggest that for some sponge species the water temperature at the time of the predator attack may be another important factor that influences sponge recovery after partial predation. The good survival of *P. croceus* transplanted during each season (before the toxic algal bloom for the summer transplant) and of explants harvested at different times indicates that the effect of water temperature on sponge recovery and survival after damage, either artificially produced or by predation, will vary between species.

7.3. Metabolite biosynthesis of sponges

Although the ecological role of most bioactive metabolites from sponges and other marine organisms is unknown (Hay 1996), several studies have suggested that sponge metabolites are used to aid in competitive interactions or prevent predation or surface overgrowth by fouling organisms (e.g., Walker et al. 1985, Bakus et al. 1990, Green et al. 1990, Chanas et al. 1996, Becerro et al. 1997a). A few studies also suggest that metabolite biosynthesis in sponges varies with location and season. For example, Thompson et al. (1987) discovered that explants of *Rhopaloeides odorabile* produce more diterpenes when transplanted to shallow depths where they are exposed to intense light, probably this prevents surface overgrowth by fouling algae which are more common at shallow depths.

Turon et al. (1996) found that the peripheral tissue of *Crambe crambe* is more bioactive in summer to repel neighbouring, competing organisms that are more common in this season. Variation in bioactivity was also found in this study. For example, farmed *L. brevis* and *P. croceus* were more bioactive than wild sponges, while an experiment done to develop suitable farming structures discovered that bioactivity of both species varied greatly with method and with substrate materials. These experiments indicate that metabolite biosynthesis in *L. brevis* and *P. croceus* is not constant but instead varies in response to different environments. In the examples above, *L. brevis* and *P. croceus* probably increased synthesis of metabolites in response to tissue damage when sponges were cut to make explants or in response to unfavourable conditions. This variation in bioactivity may indicate an optimal defence strategy, which proposes that because of an associated cost, the organism will only produce the defence, such as bioactive metabolites, when most required (Rhoades 1979, Fagerström et al. 1987, Becerro et al. 1997b).

The optimal defence strategy also has important implications for sponge aquaculture for metabolite production. For example, Munro et al. (1999) farmed explants of the biomedically important sponge *Lissodendoryx* n. sp. and found that yield and thus biosynthesis of halichondrins varied between sites and with depth. Halichondrin production was also lower in farmed sponges than in wild sponges. The variation in metabolite biosynthesis found in their study indicates that to maximise metabolite yield it is important to determine what factors influence biosynthesis and then develop a farming strategy to capitalise on this knowledge. For example, Walker et al. (1985) discovered that *Aplysina fistularis* exudes 10-100 times more bioactive metabolites than normal after experimental damage. Therefore, target metabolite yield from some farmed sponges may be increased if the sponges were damaged prior to harvesting.

7.4. Commercially farming sponges for metabolite production

This study represents the first intensive examination of sponge aquaculture for metabolite production. It determined what effect the environment has on the growth, survival and metabolite biosynthesis of the target sponges and also developed farming structures that could be used to grow sponges for metabolite production. Obviously, there is still much to be learned but when a metabolite from a sponge is successful in all clinical tests and becomes a drug, the indications are that aquaculture can be considered an important method of supply.

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Appendices

1. Farming sponges for the production of bioactive metabolites

2. The discovery and development of marine compounds with pharmaceutical potential

FARMING SPONGES FOR THE PRODUCTION OF BIOACTIVE METABOLITES

A.R. DUCKWORTH, C.N. BATTERSHILL, D.R. SCHIEL AND P.R. BERGQUIST

Duckworth, A.R., Battershill, C.N., Schiel, D.R. & Bergquist, P.R. 1999 06 30: Farming sponges for the production of bioactive metabolites. *Memoirs of the Queensland Museum* **44**: 155-159. Brisbane. ISSN 0079-8835.

For successful aquaculture of sponges, with the aim of producing metabolites, a farming method is required that promotes sponge growth and survival, and produces high yields of target metabolites. To help develop a suitable farming method growth and survival were compared for two New Zealand sponges, *Latrunculia brevis* (Ridley & Dendy) and *Polymastia croceus* (Kelly-Borges & Bergquist), experimentally grown in a variety of ways. Explants were farmed in mesh, on rope, and with rope threaded through them. For both species of sponge, survival was greatest for explants farmed in mesh, probably because this produces little tissue damage and prevents explants from dislodging and 'escaping'. This method also promoted highest growth of *L. brevis*, with some explants doubling their weight in two months. The growth of *P. croceus*, however, was highest in explants with rope threaded through them. Explants of both sponges farmed on rope did not attach and had poor growth and survival. These findings are a major step forward in developing a method for farming sponges in temperate waters of New Zealand. ❐ *Porifera, aquaculture, farming method.*

Alan R. Duckworth (email: a.duckworth@niwa.cri.nz), University of Canterbury/NIWA Centre of Excellence in Aquaculture and Marine Ecology, PO Box 14901, Kilbirnie, Wellington, New Zealand; Christopher N. Battershill, National Institute of Water and Atmospheric Research (NIWA), PO Box 14901, Kilbirnie, Wellington, New Zealand; David R. Schiel, Zoology Department, University of Canterbury, Private Bag 4800, Christchurch, New Zealand; Patricia R. Bergquist, Zoology Department, University of Auckland, Auckland, New Zealand; *24 March 1999.*

A major obstacle facing sponge aquaculture in the production of metabolites is the lack of a suitable farming method or on-growing structures (Shimizu, 1995; Osinga, 1998). To be suitable for large scale commercial use a structure must be inexpensive, have a low surface area to reduce drag and bio-fouling, and allow cost-effective and efficient harvesting. It must also promote high sponge growth and survival while also maintaining high metabolite production.

Farming structures used to grow bath sponges have historically involved attaching explants to concrete discs, or threading wire through explants so that they hang in mid-water (Cotte, 1908; Moore, 1908; Crawshay, 1939). This last method was modified slightly by Verdenal & Vacelet (1990), who successfully grew commercial bath sponges by first threading plastic-coated metal wires through explants and then attaching them to vertical ropes. Development of new farming structures to grow bath sponges was constrained by market forces determining acceptable shape and size of products (Storr, 1964; Bergquist & Tizard, 1969). In contrast, explant shape has no bearing on efficient metabolite production, and consequently there is considerable flexibility in the development of new farming structures for metabolite aquaculture.

We identified three general farming methods: 1) explants placed in mesh; 2) explants attached to and farmed on rope; 3) explants farmed with thin rope threaded through them (Fig. 1). The first method has already been tested with some success (Duckworth et al., 1997). For each method of farming it was necessary to test variation in structures and materials used. For example, rope thickness and composition were important considerations using methods 2 or 3 - as rope thickness increases, drag pressure as well as capital cost increases accordingly, whereas a decrease in rope thickness produces a decrease in available surface area for explant attachment. Rope composition is also important, because explant growth, survival and metabolite concentration may differ between ropes made of different materials.

In this study, we tested the potential of each farming method using two New Zealand sponges: *Latrunculia brevis* (Ridley & Dendy, 1886), a green massive sponge found throughout New

Zealand waters usually in exposed areas (Battershill & Bergquist, 1999a), and *Polymastia croceus* (Kelly-Borges & Bergquist, 1997), a common orange massive sponge. Both sponges contain metabolites with potential pharmaceutical properties (Lill et al., 1995; National Cancer Institute, personal communication).

The results described here are preliminary and part of a larger, ongoing experiment (October 1998). We focus here on the overall patterns of explant growth and survival between the three farming methods tested. Full results will be published after all relevant experiments are completed.

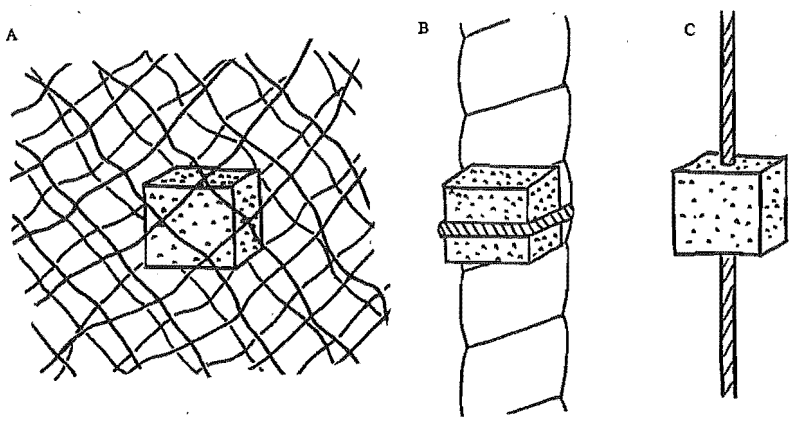

FIG.1. Schematic drawing of the 3 farming methods tested. A, explants placed in mesh; B, explants attached to rope; C, explants with thin rope threaded through them.

MATERIALS AND METHODS

For both *L. brevis* and *P. croceus*, we collected approximately forty sponges of similar size at 10-20m depth off the coast of Wellington (41°21S, 174°50E), situated at the southern end of the North Island of New Zealand. These sponges were cut, leaving approximately 30% of the original sponge intact to regenerate. Cut sponges left *in situ* had high survival and quickly healed. All collected sponges were cut under running seawater in a laboratory into cube-shaped explants, approximately 27cm^3 in size and 16g in weight. All explants had at least one side uncut, with the pinacoderm intact.

Three farming methods were tested for each species. Explants were: 1) placed in mesh; 2) attached directly to thick rope; 3) or had thin rope threaded through them (each method has several sub-methods, but full analysis at this stage is not yet possible given that the experiment is still in progress) (Fig. 1). Under method 2, each explant was firmly secured with cotton thread to an individual length of rope measuring 15x2.5cm. All explants in this method had their uncut side (with intact pinacoderm and oscules) facing outwards, away from the rope. Under method 3, to thread thin rope through explants, we carefully pushed a large needle, with rope attached, through each explant. Rope used in this treatment was 2-3mm thick. We used 40 explants of each species for each method. Explants were randomly selected and tied at intervals of 15cm to a rope back-line, and farmed at a depth of 12m.

We farmed *L. brevis* and *P. croceus* in Wellington Harbour from October 1997 to January 1998, and compared explant growth and survival. Growth was determined by wet-weighing the explants (to 0.1g) at the start and at the end of each experiment. We discovered that explants disturbed 30mins before weighing would expel all excess water, allowing us to weigh their true tissue weight.

Comparisons between the different methods of farming on growth and survival in *L. brevis* and *P. croceus* were made using one-way ANOVA.

RESULTS

In both species growth rates were not significantly different between the three farming methods tested (F_{df2}=0.24 and 0.04, N=68 and 110, P>0.05, for *L. brevis* and *P. croceus*, respectively). Conversely, survival of explants was significantly different between the methods used (F_{df2}=31.28 and 23.79, N=120, P<0.001, respectively) (Figs 2B,D). Survival of both *L. brevis* and *P. croceus* farmed in mesh, under method 1, was excellent. Only one of the forty explants of *L. brevis* died and all *P. croceus* survived. The growth of *L. brevis* explants farmed in mesh was relatively good with an average weight gain of 1.2g over the 95 days of experimentation (Fig. 2A). Some of these replicates doubled their weight from 16g to over 32g during this period, a promising result given the brief time of experimentation. Many of these explants grew through the mesh, incorporating it into their tissue. In comparison, average growth of *P. croceus* farmed in mesh was poor, increasing only 0.1g in weight over 95 days (Fig. 2C).

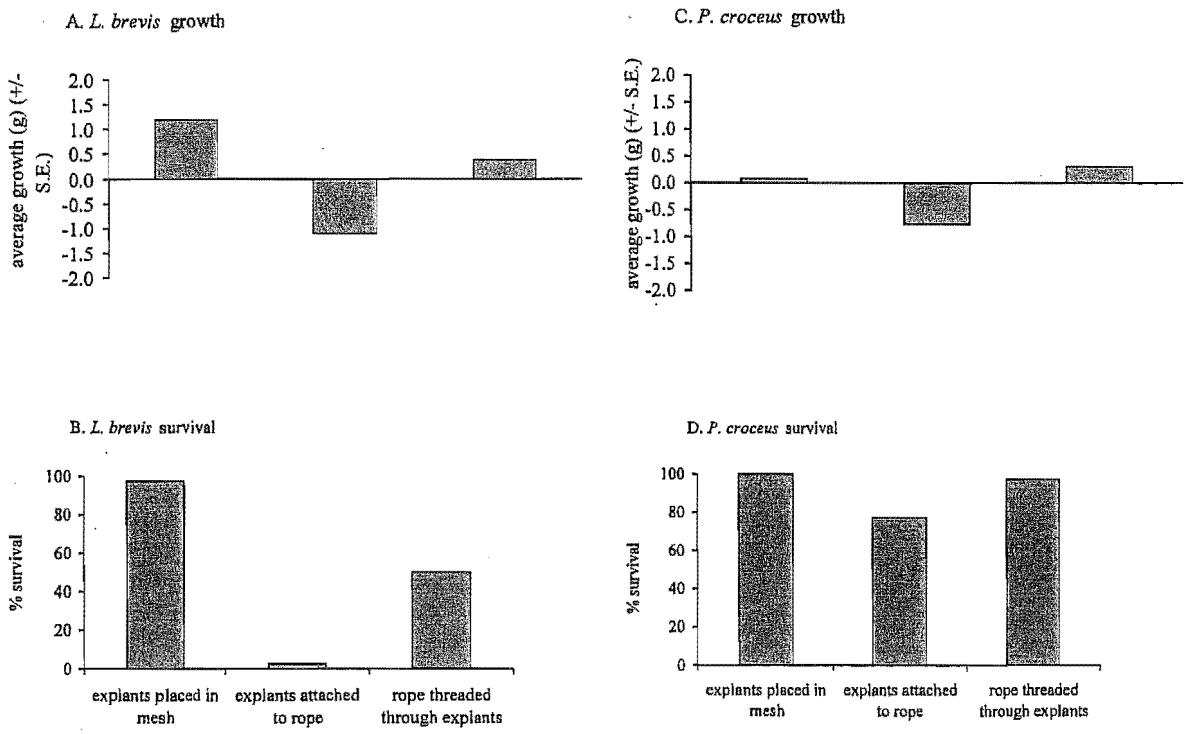

FIG. 2. Comparison in growth and survival of *L. brevis* and *P. croceus* between the three farming methods tested. Growth represents average explant weight gain or loss (+/- S.E.) over 95 days. Survival represents percent survival of the forty explants transplanted in each farming method.

Neither species grew well on rope (method 2). On average, *P. croceus* lost 0.8g while *L. brevis* lost 1.1g over 95 days (Figs 2A,C). Under method 2, survival on rope was also poor. *Polymastia croceus* had 78% survival but only 1 of 40 *L. brevis* explants survived (Figs 2B,D). Under this farming method no explants of either species attached to the rope. The explant side, in contact with the rope, was similar in appearance (morphology and colour) to the other healed sides. We also observed many explants moving or growing away from the rope, ultimately becoming dislodged.

Under method 3, when rope was threaded through explants, all but one *P. croceus* survived the 95 days experiment, whereas only 50% of *L. brevis* survived (Figs 2B,D). Average weight gain for both sponges was similar, approximately 0.3g (Figs 2A,C). Few explants of either species attached to the threaded rope. After 95 days, most explants had changed shape and were moving away from the rope.

DISCUSSION

The importance of choosing a suitable method of farming for sponge aquaculture is well demonstrated in this study. Survival of two species of sponges was greatly affected by the method used. Average growth of both species was generally low for all methods, most probably due to the short (95 day) period of experimentation, and factors inherent to each method mentioned below.

The high survival of *P. croceus* and *L. brevis* farmed in mesh (method 1) may be explained by two factors: 1) Explants experienced the least initial damage, as they are simply placed in mesh. By comparison, explants grown under the other methods had greater disturbance, with rope either pushed through or squeezed around them, causing tissue damage and increased mortality; 2) Even in cases where mesh method is not ideal, explants were effectively trapped in mesh. We noticed many explants in the rope methods moving or growing away from the rope, ultimately becoming dislodged. For farming this is effectively the same as mortality (i.e. the sponge is lost).

One disadvantage of the mesh farming method is a higher rate of fouling of mesh by sediment and sessile organisms, particularly bryozoans, reducing water flow and possibly influencing

poor explant growth or even weight loss (Bakus, 1968; Duckworth et al., 1997). Restricted water movement due to fouling probably caused poor growth of *P. croceus* farmed in mesh. Unlike *P. croceus*, many explants of *L. brevis* quickly grew through and over the mesh, reflecting inherent species differences. This reduced the effect of fouling and, combined with low explant stress and damage, probably explains the better growth of *L. brevis* farmed in mesh. Harvesting sponges growing in mesh would involve cutting away tissue growth, leaving the explant behind to grow back through the mesh.

Sponges farmed with rope threaded through them (method 3) were less effected by fouling because they were directly exposed to water. Whereas this may have promoted growth, mortality may have increased because of increased tissue damage. It is likely that increased tissue damage and rejection of the threaded rope caused poor survival of *L. brevis*. In contrast, *P. croceus* farmed with threaded rope survived well. Differences in growth and survival between the two sponges suggest that *P. croceus* is a hardier species and more amenable to different farming methods. However, given a suitable method, *L. brevis* achieved the best combination of growth and survival.

Neither species attached well to the threaded rope, which probably caused reduced growth. Other studies have shown that only explants attached to their fastening wire or identification tag grew well (Verdenal & Vacelet, 1990). The ability of sponges to change shape (Bond & Harris, 1988; Bond, 1992) allows them to move away from unpleasant conditions and can result in loss of explants and low overall survival. This farming method will not succeed unless a rope material is found to which explants will attach. We are currently investigating this, testing explant growth, survival and attachment on threaded rope made of different natural and artificial materials. It is unlikely that this farming method will be suitable in exposed areas where high water movement can easily tear sponges away from the rope.

Many studies have shown that sponges will attach well to a wide variety of natural and artificial substrata (Cotte, 1908; Moore, 1908; Crawshay, 1939; Wulff, 1984, 1985, Barthel & Theede, 1986; Bond & Harris, 1988; Rosell & Uriz, 1992). Unfortunately, both species of sponge in our study failed to attach to any of the ropes tested, perhaps a result of high substrate selectivity shown by some sponges (Battershill & Bergquist, 1999b).

Differences in growth and survival observed in the two species, *L. brevis* and *P. croceus*, probably point to inherent differences in sponge species ability to be successfully farmed. Thus, the findings of this study do not preclude the possibility of farming other New Zealand sponge species on rope. It may be possible to modify this method of farming to improve sponge attachment. For example, Battershill & Bergquist (1999b) discovered that *P. croceus* settles preferentially on rock chips, and it may be possible to incorporated these into the warp of a rope to promote explant attachment. Various types of rope substrate should also be tested.

Many factors have to be considered in the development of a method or on-growing structure suitable for farming sponges for metabolite production. These include cost, bio-fouling, harvesting procedures, explant growth and survival, and metabolite yield. The findings of this study, which concentrated on explant growth and survival using three farming methods, will help develop a suitable on-growing structure for farming massive sponges, such as *P. croceus* and *L. brevis*.

ACKNOWLEDGEMENTS

We would like to thank the many divers that helped in this study, in particular Chris Woods, Pete Notman and Phil James.

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POLLY WANT A SPONGE? : FIELD EXAMINATION OF SPONGIVORY BY CARIBBEAN PARROTFISHES IN REEF AND MANGROVE HABITATS. *Memoirs of the Queensland Museum 44: 160. 1999*:- Caribbean sponge species such as *Xestospongia muta* frequently display linear grazing scars that appear to have been made by parrotfishes, yet there are few scientific reports of parrotfish spongivory. We used a video camera to monitor 40 specimens of *X. muta* for a minimum of 0.5 hr/sponge to determine the frequency of parrotfish bites on this species. Ten hours of taping captured 45 bites on normally coloured sponges, and 527 bites on four bleached sponges. Also, the guts from parrotfishes collected in mangrove and reef habitats were digested in nitric acid and analysed for spicule content. Parrotfishes collected in the mangroves (*Sparisoma aurofrenatum*, *Scarus croicensis*, and *Sc. taeniopterus*) had a significantly greater mass of spicules in their guts than did parrotfishes collected on the reef (*Sp. aurofrenatum*, *Sp. viride*, *Sp. chrysopterum*, *Sc. vetula*, *Sc. coelestinus*, and *Sc. taeniopterus*). Up to 148mg of spicules were present in the guts of mangrove parrotfishes. The spicules of *Geodia gibberosa*, a sponge that is common in the mangroves but rare in exposed locations on the reef, were abundant in the gut samples. Our results suggest that some sponge species are palatable not only to specialist predators such as sea turtles and angelfishes, but also to species that are not usually recognised as sponge predators. ❐ *Porifera, spongivory, parrotfishes, Xestospongia muta, Geodia gibberosa, Sparisoma spp., Scarus spp., spicules, ecology, predation.*

Matthew J. Dunlap (email: dunlapm@hotmail.com) & Joseph R. Pawlik, Department of Biological Sciences, University of North Carolina-Wilmington, 601 South College Road, Wilmington NC, 28403-3297 USA; 1 June 1998.

DEVELOPMENT OF *HALISARCA DUJARDINI* JOHNSTON 1842 (PORIFERA, CERACTINOMORPHA: HALISARCIDA) FROM EGG TO FREE LARVA. *Memoirs of the Queensland Museum 44: 160. 1999*:- Embryonic development in the sexual viviparous sponge *Halisarca dujardini* from the White Sea (Arctic) shallow water was studied. Complete, equal, asynchronal cleavage is characterised with variability of analogous developmental stages and the lack of the strictly determined clevage spindles position. The cytoplasm is filled with numerous yolky granules with heterogenic contents. At the 16-24 cell-stage a small cavity is formed. Blastomeres and the embryo polarity are not expressed. Large nuclei containing pronucleolar bodies are situated at the central parts of the cells. From the 16-24 cell- stage, true nucleolus formation starts. The polarisation of blastomeres is expressed by the distal movement of nuclei and changes in cell form. Cleavage furrow planes obtain the similar radial pattern forming roundish coeloblastula 130-170μm in diameter with the small cavity restricted with long wedge-shaped cells.

The internal layer of the larva is formed at the 100-130 cell-stage owing to the individual cells' apolar migration out of the blastula walls. At the same time flagella are formed on the cells' apical surfaces, yolk granules being concentrated basally. Internal cells proliferate actively, differentiating into nucleolated amoebocytes, granular cells and collencytes.

The larvae are is disphaerula it consists of two flagellated sphaerae external and internal. The disphaerula is completely covered with flagella.

Flagellated cells are less numerous at the posterior pole. Flagellated epithelial cells are wedge-shaped. At their apical parts they contain a drop-like nucleus with nucleolus and a flagellum embedded into a pocket-like cytoplasmic invagination. The basal 2/3 of the cell volume is filled with numerous yolk granules. Flagellated cells are connected at their apical end by outgrowths of the plasma membrane embedded into similar invaginations of the neighbouring membrane. Posterior flagellated cells are trapeziform or rectangular, and contain numerous yolk granules. The nuclei are roundish, with large nucleoli. The internal sphaera is formed by invagination of lateral cells. These sphaera are formed by a layer of cylindrical cells that have flagella inside the cavity. Their piriform nuclei contain nucleoli, and there are yolk granules in the cytoplasm. There are no specialized cell contacts between blastomeres and larval cells. The spiral symbiotic bacteria are present in the central part of the larva and in intercellular spaces. Some peculiarities of *H. dujardini* embryogenesis are unique among Ceractinomorpha and are a matter of principle for comparative embryological studies of Porifera. They are: 1) total equal asynchronic cleavage; 2) equal, apolar coeloblastula with a small cavity; 3) unexpressed polarity of blastomeres; 4) subsequent of the same type radial cleavage leading to the cell polarisation and coeloblastula formation; 5) formation of an internal cell mass in the embryos by multipolar cell ingression at the 100-130 cell-stage; 6) development of special larva disphaerula; 7) formation of internal sphaera by invagination. All the features mentioned can serve as additional arguments for separation of the Halisarcida as an order (Bergquist, 1996). ❐ *Porifera, Halisarca dujardini, embryology, cleavage, larva, cells, ultrastructure.*

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Journal of Biotechnology 70 (1999) 15–25

JOURNAL OF Biotechnology

The discovery and development of marine compounds with pharmaceutical potential

Murray H.G. Munro [a,*], John W. Blunt [a], Eric J. Dumdei [a], Sarah J.H. Hickford [a], Rachel E. Lill [a], Shangxiao Li [a], Christopher N. Battershill [b], Alan R. Duckworth [b]

[a] *Department of Chemistry, University of Canterbury, PB 4800, Christchurch, New Zealand*
[b] *National Institute of Water and Atmospheric Research, PO Box 14-901, Wellington, New Zealand*

Received 12 October 1998; received in revised form 1 December 1998; accepted 22 December 1998

Abstract

An assessment of the current status of marine anticancer compounds is presented along with a case study on the aquaculture of *Lissodendoryx* n. sp. 1, a sponge that produces the antimitotic agents halichondrin B and isohomohalichondrin B. The use of polymer therapeutics to enhance the properties of marine natural products is considered.

Keywords: Biodiversity; MarinLit; Sponge aquaculture; Anticancer; Antimitotic; Polymer therapeutics

1. The discovery phase

In contrast to work on terrestrial natural products the first serious work on studying marine natural products started just 50 years ago with the pioneering work of Bergman (e.g. Bergman and Feeney, 1951). While the difficulties of collecting marine samples cannot be underestimated, a large number of easily accessible marine samples are available simply by shore-wading. That the opportunity was not seriously grasped until the 1940s is possibly a commentary on the difficulties of isolation and purification of marine natural products with the limited techniques available at that time. However, since the 1940s the field has blossomed and matured. In 1997 there were 713 papers published on marine natural products. This is out of a total of 10311 papers recorded in MarinLit, a database dedicated to the marine natural products literature (MarinLit, 1998). At the time of the mid-year release of the 1998 version of MarinLit, 484 new papers had been included. From the marine literature it is the Porifera that have been the most studied phylum followed closely by the Cnidaria, Chromophycota, Rhodophycota, Mollusca, Chordata and the Echinodermata (MarinLit, 1998).

* Corresponding author. Tel.: +64-3-364-2434; fax: +64-3-364-2110.
E-mail address: m.munro@chem.canterbury.ac.nz (M.H.G. Munro)

0168-1656/99/$ - see front matter
PII: S0168-1656(99)00052-8

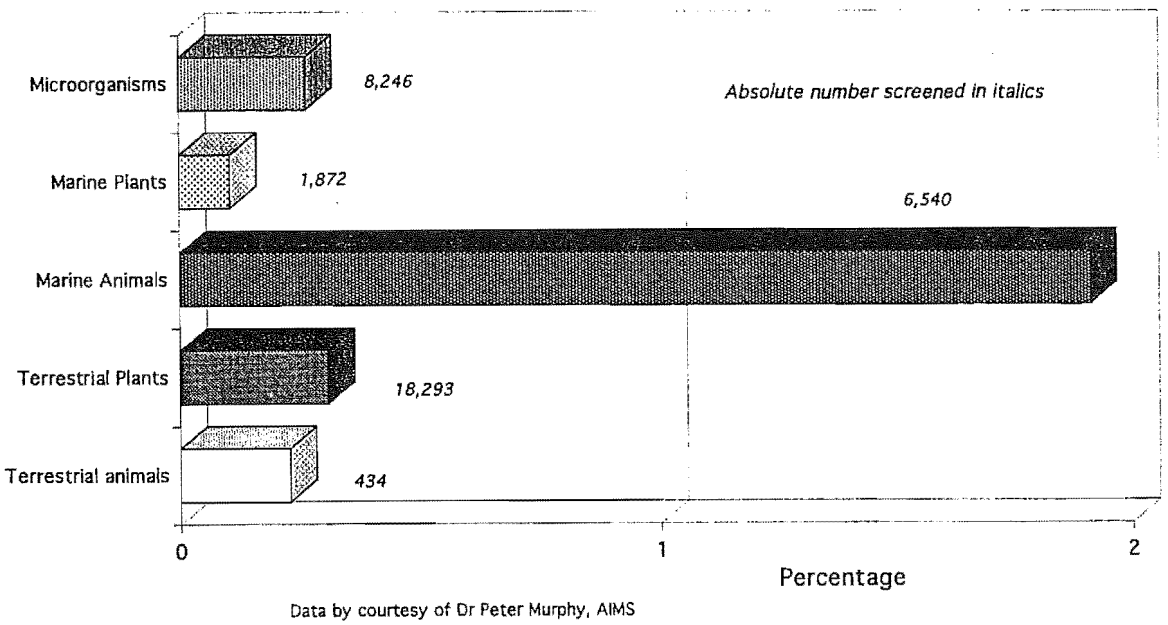

Fig. 1. Distribution of samples with significant cytotoxicity in the NCI's preclinical screen.

Over the years some distinct trends have emerged in the study of marine natural products. One has been the emphasis on the discovery of new bioactive natural products. Initial work by Bergman was undoubtedly curiosity-driven, but it was his discovery of the biologically-active, pharmaceutically important and novel arabino-nucleosides from the sponge *Cryptotethya crypta* that sparked interest in marine natural products and served to highlight the biomedical potential of the field (Bergman and Feeney, 1951). With advances in chromatographic techniques for dealing with polar compounds along with better analytical and structural elucidation technology an increasing proportion of the compounds isolated have shown cytotoxic properties (suggestive of potential antitumour compounds). In an early review (Munro et al., 1987) covering the marine literature up to early 1986, 185 bioactive compounds were reported. In 1993 a review (Schmitz, 1994) covering the next 5 years commented on an additional 400 compounds. A survey of MarinLit reveals that this trend has continued with some 46% of all cytotoxic compounds in the database having been reported since 1993.

As a source of bioactive compounds with pharmaceutical potential how well does the marine environment compare with the more traditional areas such as terrestrial microorganisms and plants? The best comparative data is that published by Garson based on statistical data from the US National Cancer Institute (NCI) screening programme provided by Dr Peter Murphy. This clearly indicated that marine invertebrates are a preferred source due to the much higher incidence of *significant* cytotoxic activity (Garson, 1994) (Fig. 1). If those screening data for marine animals are in turn examined on a phylum basis certain phyla (e.g. Porifera, Bryozoa, Chordata) have a higher incidence of bioactivity with the trend becoming very obvious as species with very significant bioactivity ($IC_{50} < 2$ μg ml^{-1}) are selected (Fig. 2).

As the data in Fig. 1 suggest, the sampling of oceanic life-forms enhances the probability of discovering species from natural sources with potential anticancer activities. This can be rationalised as a sampling strategy which accesses the widest range of phyla. Greater than 70% of all recorded living species belong to the animal kingdom.

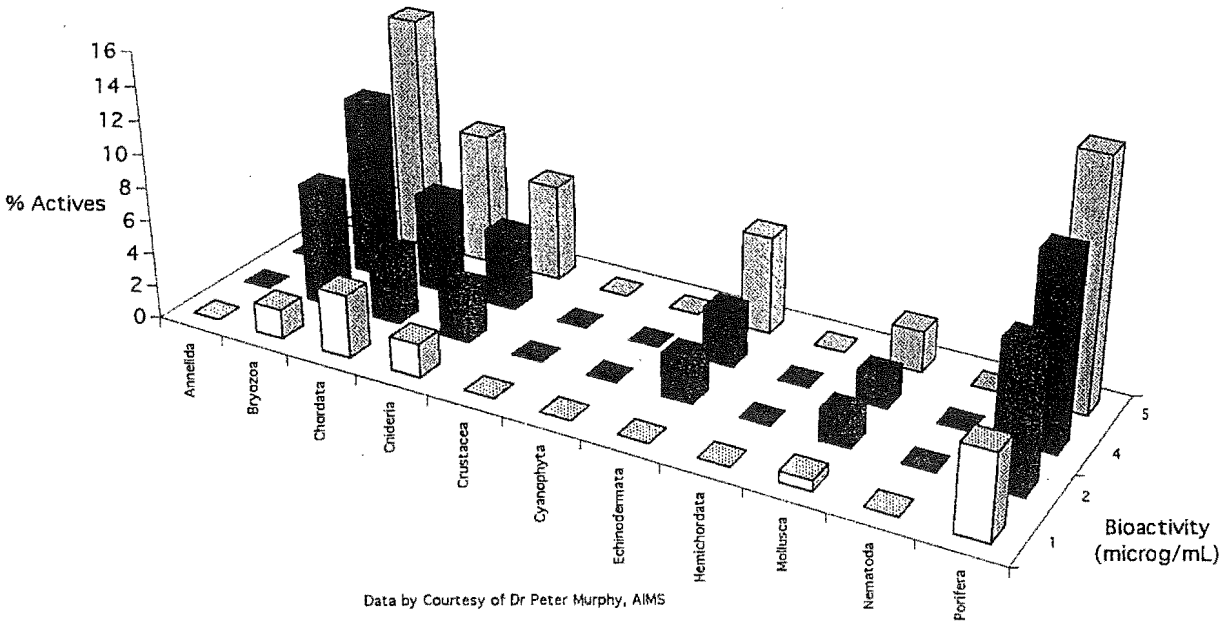

Fig. 2. Cytotoxicity by phylum.

While only 28% of the animals are aquatic these in fact represent >90% of the animal phyla (May, 1988). When searching for bioactivity the total number of samples collected is important, but it is not as important as sampling across phyla. Put simplistically, the probability of finding a bioactive species (a 'hit') can be expressed as:

'Hits' ≈ Samples × 'Biodiversity of Samples' × Assays

Clearly, the greater the number of samples assayed in the greatest number of possible assays will enhance the probability of finding useful compounds. With the advent over the last decade of high throughput screening (HTS) the number of assays that can be applied to any sample has gone up by between one and two orders of magnitude. However, the key term in the expression is 'biodiversity of samples'. This is a qualitative factor relating to the number of unique structural classes of compounds sampled (adapted from Devlin, 1997). By sampling across phyla the probability of finding unique classes of compounds is higher than by sampling many species within one phylum. The factor associated with 'biodiversity of samples' is probably higher for a random collection of marine organisms than from any other source and perhaps explains why marine samples offer greater opportunity for the discovery of unique compounds with pharmaceutical potential.

2. Current status of bioactive marine natural products

In spite of the advances in computer-assisted drug design, in molecular biology and gene therapy there is still a pressing need for new drugs to counteract drug-resistant pathogens like, for instance, the mycobacterium that causes tuberculosis, or multi-drug resistant cancers, or even disease states such as Alzheimers which is of pressing concern as the age demographics of the Western World change. What of value has emerged from the marine resource over the past 50 years that would justify the investment of time and money and optimism in this field? In the area of cancer there have been valuable discoveries. Likewise in the area of inflammatory diseases (Fenical, 1996), while probably the first marine-based drug that will be marketed is in the area of

intractable pain management (Olivera, 1997). However, for the purposes of this overview only the cancer leads will be commented on.

Progress in the cancer area is summarised in Table 1. Of the four compounds now in clinical trials two are derived from tunicates. These are ecteinascidin 743 and aplidine (dehydrodidemnin B). The other compound, dolastatin 10, originated from a bryozoan. Didemnin B, another tunicate-derived compound closely related to aplidine, has previously been in clinical trials and reached as far as phase 2 as had bryostatin 1 before being withdrawn in late 1998.

At a preclinical phase are halichondrin B, a sponge metabolite, and kahalalide F from a mollusc followed by a range of compounds from a variety of sources, but all with potent in vivo activities against a range of cancer cell lines. From the mechanism of action of these marine metabolites it is very obvious that the marine environment has been an excellent source of antimitotic agents. Cancer chemotherapy exploits differences between normal and malignant cells. Ideally, total selectivity between the cell types is required, but has not been achieved. The high proliferation rate of cancer cells is one area that is targeted when looking for cytotoxic agents. Compounds that block mitosis (anti-mitotic agents) which occurs during cell proliferation have become some of the most important anticancer agents, e.g. taxol, vincristine. Antimitotic agents can be divided into four categories depending on which particular step in the microtubule polymerisation/depolymerisation steps that they inhibit (as indicated in Table 2) (Avila, 1997).

Two of these antimitotic agents are already in clinical trials and many of the others listed are being actively investigated as potential anticancer agents. Undoubtedly, some will proceed further to clinical trials. There seems little doubt now that one, or more, of these potent marine natural products (or synthetic analogues derived from them) will emerge in the future as new, therapeutic agents in the fight against cancer.

Table 1
Testing status of anticancer marine metabolites

Status	Compound (origin/activity)
Clinical	–
Phase III	–
Phase II	Ecteinascidin 743 (tunicate/antimitotic)
Phase I	Aplidine (dehydrodidemnin B) (tunicate/protein synthesis inhibitor) Dolastatin 10 (sea hare/antimitotic)
Pre-clinical	Halichondrin B (sponge/antimitotic) Kahalalide F (mollusc/ -)
In vivo active	Aplyalronine (sea hare/actin) Thiocoralline (marine microorganism/RNA inhibition) Isohomohalichondrin B (sponge/antimitotic) Discodermolide (sponge/antimitotic) Sarcodictyins (soft coral/antimitotic) Eleutherobins (soft coral/antimitotic) Spongistatins/Altohyrtins/Cinchyrolide (sponges/antimitotic) Lamellarin N (mollusc/tunicate/sponge/antimitotic) Cryptophycins (blue green alga/antimitotic)

Table 2
Marine-derived antimitotic agents

Type	Marine compound
Type I (colchicine site)	–
Type II (GTP binding site)	Halichondrin B Isohomohalichondrin B Spongistatins/Altohyrtins/Cinchyrolide Cryptophycins Dolastatin 10
Type III (microtubule stabilisation)	Eleutherobin Sarcodictyins Discodermolide
Type IV (microtubule network disorganisation)	Ecteinascidin 743 Lamellarin N

Table 3
Potential supply sources of the marine-derived anticancer compounds

Natural sources	Aquaculture/fermentation	Synthesis	Synthesis?[a]
Didemnins	Halichondrins	Ecteinascidins	Halichondrins
Ecteinascidins	Cryptophycins	Cryptophycins	Eleutherobins
Halichondrins	Thiocoralline	Dolastatin 10	Sarcodictyins
		Lamellarins	Discodermolide
			Aplyronines
			Spongistatins/
			Altohyrtins/
			Cinchyrolide

[a] The synthesis has been achieved, but yields and number of steps not yet applicable to production.

3. The development phase

Given the difficulty in synthesising many of the marine natural products, perhaps the most significant hindrance to their development as drugs, or industrial biocides, is their limited supply. The marine resource offers the biological diversity for sampling in the discovery-phase of new drug development. What is less attractive about marine macroorganisms is the lack of knowledge about obtaining either the organism in bulk, or sourcing the key compound by a routine method. There is no routine, easy source of material for scale-up such as seeding out plantations, or fermentation on a 50 000 L scale as applies to plant, or microbial products. No matter how attractive a biological profile a compound might possess, unless an adequate supply stream can be generated the compound will remain of novelty value only. For example, at the NCI if an adequate initial supply of the compound can be obtained an in vivo active compound can proceed as far as Decision Network IIA (DNIIA), an advanced point in that organisation's preclinical evaluation of anticancer compounds, but unless arrangements can then be made for the purchase or supply of the compound in bulk the compound will not proceed to the next step, DNIIB. Halichondrin B is a good example of a compound in that situation.

When a marine natural product succeeds in the development phases, techniques for large-scale commercial supply need to be employed immediately in order to maximise the patent, or license investment. Realistically, the bulk supply of bioactive compounds can only be achieved by harvesting from natural origins, by aquaculture/fermentation, or by synthesis. Neither tissue culture nor genome transfer from the producing organism to an appropriate vector can be considered as viable supply options at least for the foreseeable future. All but one of the compounds listed in Table 1 have been synthesised (kahalide F), but one has to distinguish between an 'academic' synthesis and an 'industrial' synthesis. The former is where the goal is simply to synthesise the compound, preferably being the first to do so, and by a novel and elegant route. In the latter case the aim is to provide a viable, low-cost synthesis with as few steps as possible. Not all compounds lend themselves readily to synthesis on a large scale. This is usually due either to the complexity of the skeleton, the number of stereogenic centres, or a combination of both. Other compounds, like those of peptide origin, are more amenable to synthesis and compounds such as dolastatin 10 fall into this category. Supply from natural sources by careful harvesting works well for rapidly growing and abundant species. One example of this is the supply of the tunicate-based antimitotic ecteinascidin 743. In other cases where the source organism is rare, or grows only in extreme conditions, then aquaculture might be the only way of obtaining sufficient compound. When an assessment[1] is made of the potential supply source of the current antitumour compounds (Table 1) the following pattern emerges (Table 3).

[1] A personal judgement specialist (MHGM and JWB) assisted by Dr Johnathan Morris, a special synthetic chemist.

Of the compounds in clinical trials, adequate supplies have been obtained from natural sources by harvesting with good husbandry practices, by aquaculture, or by total synthesis. Of the candidates at the pre-clinical phase it would be possible to obtain adequate supplies of halichondrin B by aquaculture methods. This is the first time that a Porifera secondary metabolite has become accessible by aquaculture. The same comment also applies to the production of ecteinascidins and bryostatins, metabolites from the phyla Chordata and Bryozoa, respectively.

4. Production of halichondrin B by aquaculture

In work in New Zealand over the past 14 years ~6000 samples have been collected and assessed for biological potential. The samples have been mainly sponges and tunicates from sites ranging from Antarctica, through the sub-Antarctic islands, and around the coast line of New Zealand up to the Northern tip of the North Island. The most promising candidate discovered to date is the sponge *Lissodendoryx* n. sp. 1. The bioactive components in this sponge are a series of compounds belonging to the halichondrin B family, which have now been found in a total of four sponges (the others are *Halichondria okadai* (Hirata and Uemura, 1986), an *Axinella* sp. and *Phakellia carteri* (Pettit et al., 1991, 1993)). Halichondrin B is being assessed by the NCI, while isohomohalichondrin B, a related compound (Litaudon et al., 1994), will be developed independently in Europe by PharmaMar SA.

Lissodendoryx n. sp. 1 is a rare, deep water species (−80 to −100 m) found exclusively off the Kaikoura Peninsula (Page and Battershill, 1998). An extensive environmental survey, conducted using an ROV and a benthic camera, established that the 'sponge field' was only ~5 km^2 with the mean biomass and abundance of sponge estimated to be 69 ± 21 g m^{-2} with 1.1 ± 0.1 individuals per m^2 over the sponge field. This survey gave an estimated total biomass of the *Lissodendoryx* sponge of just 289 ± 90 t total (Dumdei et al., 1998; Page and Battershill, 1998) and established quite unambiguously that the halichondrins could *never* be supplied on a commercial scale by collection from the wild. However, based on the results of the survey a permit was obtained and a collection of 1 t made, from which the halichondrins were isolated (310 mg of halichondrin B (NCI) and a comparable amount of isohomohalichondrin B) providing sufficient mass for the initial preclinical trials only.

To establish a supply option for the halichondrins the New Zealand National Institute of Water and Atmospheric Research (NIWA), in collaboration with the University of Canterbury and the NCI, have carried out aquaculture feasibility trials at various scales on *Lissodendoryx* n. sp. 1. There were early indications that one mode of *Lissodendoryx* reproduction was by fragmentation and advantage was taken of this observation. It was established that small explants were capable of extremely rapid growth (up to 5000% within 1 month) given the correct conditions (Battershill and Page, 1996). Preliminary experiments also established that use of a scallop lantern was feasible as a support and an 'analysis of variance' model was adopted to allow examination of variability of growth and target metabolite production patterns in response to the following factors: season, location, site (within location), and depth. The experiments were run over a period of 18 months. It was quickly apparent that summer transplants were not successful and that while significant growth of the explants was observed at the deep sites this was short-lived, as all sponges succumbed to bryozoan overgrowth and pathogen attack. Mortality was over 95%. In contrast, the winter explants (April) at all sites were generally successful (mortality less than 15%), especially at the greater depths, with the same previously observed growth rates observed through to December, when a decline was observed as fouling again became excessive. The mortality rate of the sponges was high in summer, especially at shallower depths, and a critical temperature of 18°C has been identified above which the sponge will not survive (Dumdei et al., 1998).

The next most important question after getting the sponges to grow and survive was whether or not the halichondrins were being produced under these conditions. Samples of the biomass were

taken at regular intervals across all sites and extracted and examined by bioactivity assays and HPLC for the presence of the various halichondrins. From the bioactivity profiles it could be established that halichondrins were being produced and by HPLC analysis of five bulk samples from two sites at differing depths the production and profile of the halichondrin production was established. Wild samples of the sponge typically contain ~ 400 μg kg^{-1} of halichondrin B, ~ 200 μg kg^{-1} of homohalichondrin B and ~ 900 μg kg^{-1} of isohomohalichondrin B. The overall halichondrin content of the cultured sponge was not as high as that of the wild sponge, and the production of the individual halichondrins was site dependent (see Fig. 3) (Dumdei et al., 1998). The relative production of halichondrin B in the cultured samples was generally higher than that found for homohalichondrin B and isohomohalichondrin B and, depending on the site, the figure for the total production of halichondrins per kg of sponge ranged from 30 to 60% of that found in the wild sponge at four of the sites surveyed. This is a significant rate of production, especially when rate of growth is taken into account.

These initial experiments showed that the sponge could be grown successfully in small-scale trials and that the halichondrins continued to be biosynthesised, even after several years of culture. There was a need, however, to scale-up experimentation to a level that would simulate commercial production conditions. This was carried out in late 1997 using a variety of culture methods. The selection of culture methods was dictated by consideration of those approaches that could ultimately be most readily converted to a large-scale mechanised operation. Wild samples of *Lissodendoryx* n. sp. 1 were collected from the sponge field off Kaikoura and immediately transferred to aerated buckets and flown by light aircraft to Wellington before deployment. Explants (8 cm^3) were allocated at random into the following treatments: lantern, tray, bag or disc, all at 10 m depth. The lanterns used were commercially available scallop lanterns 1 m in diameter, with ten tiers supported by wire hoops and covered in a 1.5 cm nylon mesh. Tray culture represented a prototype sponge deployment system which essentially held explants in a vertically aligned nylon mesh sandwich. Bag treatments similarly represented a prototype for a continuous stocking type culture system and finally, discs held sponges in a clutch-type culture array where explants were suspended on ropes without any surrounding mesh. The explants were monitored for three months. Growth and mortality were measured in situ. Fig. 4 shows the changes in volume (directly proportional to weight in this species).

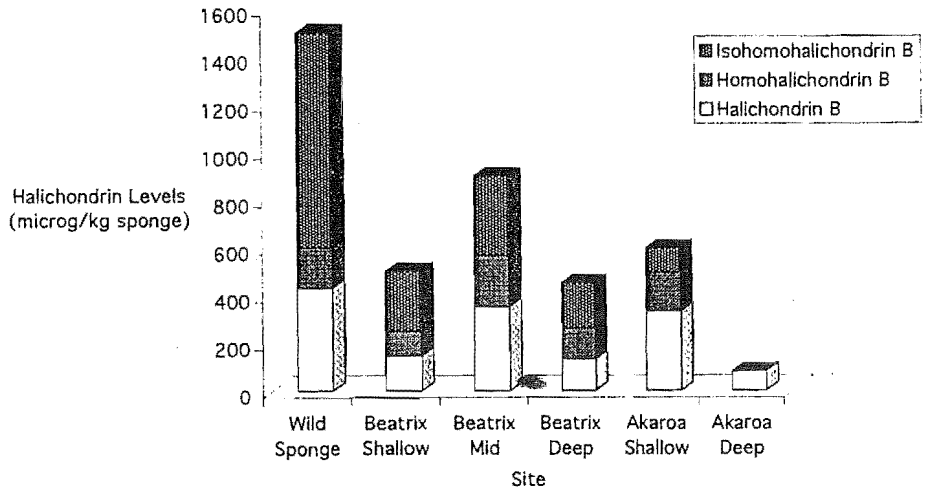

Fig. 3. Halichondrin levels in *Lissodendoryx* samples.

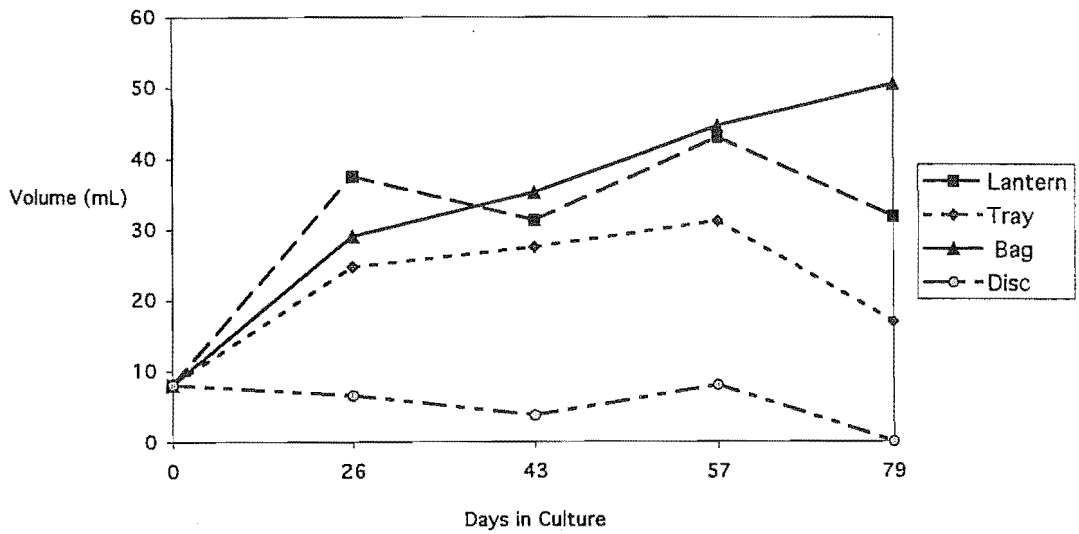

Fig. 4. Growth rate as a function of culture substrate.

Sponges cultured in trays, or on discs did not grow well. In both cases, fouling became a significant problem as the plastic component of the support structures appeared to promote settlement of fouling species, predominantly *Bugula flabellata*. The best initial growth rate was observed in lantern cultures, but after 26 days sponges were observed to lose weight and condition. It was apparent that the enclosed nature of the apparatus retarded growth after a certain size was obtained and that the lanterns also soon became severely fouled. The bag-type culture system proved to be the best both in terms of promoting fast growth, as well as maintaining growth. Explants quickly grew through the mesh and were soon able to grow uninhibited, using the overgrown bag for support. This culture system was also the most readily adaptable to a mechanised system and could be developed to a large-scale stocking-type culture system, not unlike traditional mussel-seeding aquaculture.

This suite of experiments was carried out in summer. This is usually a period through which the sponge does not grow in nature and hence represents a worst-case example of the potential of sponge aquaculture. None the less it was established that in less than 26 days sponge explants could quadruple their volume and weight. In other experiments, carried out at the same time but at different localities, explants were observed growing to 120 cm^3 in less than 30 days, clearly indicating that there will be locations which are more suitable for growing sponges (Battershill, unpublished data).

These results show that aquaculture of sponges is a viable and reliable option for creating extractable biomass. Very preliminary estimates of biomass production suggested that the annual production of 5 t of sponge per 100 m of longline is achievable (Dumdei et al., 1998). These estimates were based on use of scallop lanterns, good husbandry to reduce overgrowth, two harvests a year, mortality restricted to $< 50\%$ and growth of 8 cm^3 explants to ~ 300–400 g. The use of stocking-type systems as opposed to scallop lanterns will influence both the likely yields as well as the economics of production. The economics of production is likely to be very competitive as the mechanised technology is already available for seeding and harvest using stocking type systems. In addition, sponges may be readily grown with other species such as mussels in successful polyculture operations.

If the halichondrins progress satisfactorily through preclinical trials over the next year, gram quantities of halichondrin B and isohomohali-

chondrin B will be required. This equates to tonnes of sponge which can only be supplied by aquaculture. Harvesting at that scale from the limited wild-stock is not possible. The supply of this quantity of the compounds will give the opportunity to estimate more accurately the actual production parameters and economics of the production of *Lissodendoryx* n. sp. 1 by aquaculture. Should either halichondrin B, or isohomohalichondrin B then proceed through clinical trials and become established as pharmaceuticals the estimated amount of each compound required annually is ~5 kg. This estimate is based on the potency of the halichondrins in in vivo animal trials and projected dose regimes and corresponds to the annual production of at least 5000 t of *Lissodendoryx* n. sp.

5. Future trends

The development of a successful pharmaceutical requires that attention also be paid to delivery of the drug as well as supply. The antiproliferative properties of today's anticancer compounds will never overcome solid tumours because of the sensitivity of the surrounding tissue to the fatal effects of exposure to the compounds. This limits the use of high concentrations. What is required are alternative approaches that facilitate the specific targeting of tumours. One approach, known as polymer therapeutics, is a rapidly growing multidisciplinary field requiring the combined talents of organic chemists, polymer chemists, pharmacologists and oncologists (Duncan, 1992). The concept behind polymer therapeutics is shown schematically in Fig. 5. The drug is attached via a biodegradable linker to a water soluble polymer. In other, optional approaches, specific targeting residues can also be added.

Polymer therapeutics not only offers improved pharmacokinetic properties, but better targeting of tumour tissue and higher selectivity. The basis for this better targeting and selectivity operates by what is known as the 'enhanced permeability and retention' effect (EPR) which leads to higher concentrations of the anticancer agent within the tumour. In vivo trials have established the success of this approach. Two such drugs currently undergoing phase I/II clinical trials are PK1 and PK2 where the anticancer drug doxorubicin has been attached via a tetrapeptide linker to a water-soluble hydroxypropyl-methacrylamide (HPMA) polymeric backbone. The tetrapeptide linker was designed to resist peptidase activity in the bloodstream, but be susceptible to lysomal enzymatic hydrolysis following the transfer by endocytosis to the interior of the target tumour cells (Duncan et al., 1996).

Polymer therapeutics is an ideal approach to enhancing the value of marine toxins. Compounds like halichondrin B are already established by in vivo trials as effective agents which can be transferred intravenously to remote sites within the test animal and inhibit the growth of a range of human tumour types. Any modifications that can enhance pharmacokinetic properties, reduce required plasma concentrations and exhibit enhanced selectivity can only be considered advantageous. To this end we are working in collaboration with the NCI and the London School of Pharmacy on the development of a polymeric therapeutic based on the halichondrin skeleton. An amino derivative of the halichondrin skeleton has been synthesised and converted into a polymeric form comparable to PK1 and is currently undergoing in vitro testing against a range of human tumour cell lines.

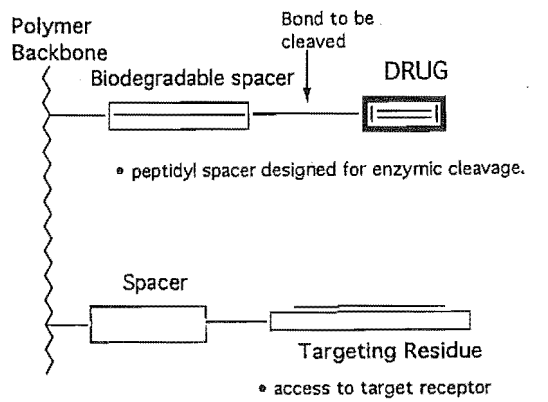

Fig. 5. Schematic showing basic features of a polymer therapeutic drug.

Ultimately, this approach will be applied to other marine toxins such as the mycalamides (Perry et al., 1988), the discorhabdins (Perry et al., 1986), pateamine (Northcote et al., 1991), calyculinamides and swinholides (Dumdei et al., 1997) that we have isolated from New Zealand marine organisms. The interest in the swinholides and calyculinamides will centre on the attempt to develop these two toxins into in vivo active polymeric drugs. Both classes of compound have potent activities in vitro, but are inactive in vivo. The swinholides disrupt the formation of actin filaments while the calyculinamides are protein phosphatase 2A inhibitors. These biological profiles complement the antimitotic properties of our initial polymeric drug based on aminohalichondrin.

6. Conclusion

The sea offers a rich source of biodiversity from which a series of potential drugs, particularly in the area of cancer chemotherapy, have already been discovered. That aspect of the drug development process is dominated by chemists, zoologists and biochemists. The development phase, where there is the pressing need for supply of these compounds, will need strong leadership from the marine biotechnologists as not all marine-based drugs will be able to be synthesised, or obtained by fermentation technology. There will be a need for new, innovative approaches to the aquaculture of many species from phyla that traditionally have not been subject to aquaculture. For those compounds that do become marketable drugs there will exist the challenge to grow the producing organisms commercially by aquaculture.

The chemists will continue to find new leads. Can the biotechnologists produce the compounds economically? That is the challenge for the future.

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